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ARCHIVES OF PATHOLOGY

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ABSORPTION OF SCAR TISSUE IN EXPERIMENTAL NODULAR CIRRHOSIS OF THE LIVER

With a Method of Visualizing Cirrhotic Changes

BERNHARD STEINBERG, M.D., and RUTH A. MARTIN, M.T.
TOLEDO, OHIO

IT IS generally accepted that the fibrosis of the cirrhotic liver is an irreversible change. It is also believed that no structural repair is possible after scar tissue has been laid down and the hepatic parenchyma subdivided haphazardly. The causes of most forms of cirrhosis in which scarring is conspicuous are not well understood, and treatment is ineffectual. The condition progresses unfavorably and terminates in death. Hence in the human type of the disease the concept of "irrevocable change" cannot be tested. The "nodular cirrhosis" produced with azo compounds by Yoshida,[1] Nakazawa[2] and Kinosita[3] offered an opportunity to verify whether scarring, at least in the experimental form of portal cirrhosis, is irreversible.

Orr[4] produced nodular cirrhosis in rats by feeding them 20 to 30 cc. of 3 per cent butter yellow (paradimethylaminoazobenzene) in olive oil per kilogram of wheat or unpolished rice, with a supplement of "green stuff." The investigator terminated the feeding of butter yellow at the end of the seventh month. He killed a number of animals monthly for a period of eleven months. He presents an excellent description of the progressive changes of cirrhosis. One must be critical, however, of his assumption that cirrhosis begins in all animals at the same time and in the same degree. Orr also noted that in many of the rats the structural pattern of the liver returned to normal after the tenth month. Although this observation suggests reversibility of the fibrotic process, it is by no means conclusive. Random selection of animals each month presupposes that the disease develops in all of them in equal degree. This supposition does not coincide with the results of our experiments.

From the Toledo Hospital Institute of Medical Research.

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4. Orr, J. W.: J. Path. & Bact. **50**:393, 1940.

In our preliminary investigations we noted the presence of normal hepatic patterns in some animals after butter yellow was withdrawn. However, the livers of other rats which died or were killed while restricted to the diet either showed patchy change or were completely normal. These observations indicated that the presence and the degree of cirrhosis must be ascertained in the living animals prior to investigations dealing with recovery. A procedure which satisfied these requirements was described by us in collaboration with Walliker.[5] A colloidal suspension of thorium dioxide was introduced intracardially in rats. The progressive cirrhotic changes could be readily visualized on roentgenograms.

EXPERIMENTAL PROCEDURES

Fifty albino rats of the Wistar strain, 3 to 5 months of age and of both sexes, were used in the experiment. The animals were given a diet of brown unpolished ground rice with butter yellow in the concentration of 0.06 per cent and 1 Gm. of carrot daily. The ration was prepared by dissolving 3 Gm. of butter yellow in 100 cc. of olive oil with gentle heating. Each 1,000 Gm. of rice was thoroughly mixed with 20 cc. of the butter yellow solution. No limitation was placed on the quantity of butter yellow–rice mixture consumed by the animals. However, their consumption of carrots was restricted. The feeding of unlimited amounts of vegetables is inadvisable for several reasons. For one, the animals consume the vegetables and do not eat the butter yellow–rice mixture.

The colloidal suspension of thorium dioxide was injected intracardially in 0.5 cc. quantities from two to four times. The first two injections were made at an interval of three months and the others after the animal was returned to its normal diet. Roentgenograms were taken of chest and abdomen prior to the feeding of butter yellow and at monthly intervals thereafter for the duration of the animal's life. Visualization of the liver and the spleen was obtained on roentgenograms as follows: Projection was anteroposterior and posteroanterior. No filter was used. The distance was 36 in., the kilovolt peak 60 and the amperage 100 ma. An Eastman no-screen film was used. The animals were under ether anesthesia.

A group of 26 animals was used in a study of the correlation of gross and microscopic changes of the liver with the roentgenographic appearances. Rats were killed at intervals of from two weeks to seven months after the start of the dietary regimen.

A group of 24 rats was allowed to acquire various degrees of cirrhosis. When the presence and the extent of the cirrhosis had been established by roentgenograms, the animals were returned to a stock diet made up as follows: cornmeal 69.64 per cent, casein 3.08 per cent, soybean oil meal 10.8 per cent, alfalfa leaf meal 2.06 per cent, wheat germ 10.8 per cent, yeast 2.06 per cent, calcium carbonate 0.52 per cent, cod liver oil 0.52 per cent and salt 0.52 per cent. At intervals of from two weeks to eight and a half months after the return to the stock ration, 2 to 4 animals were killed. The presence of cirrhosis and the degree of return to normal as determined by gross and microscopic criteria were correlated with the roentgenographic appearances.

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5. Steinberg, B.; Walliker, C. T., and Martin, R. A.: Proc. Soc. Exper. Biol. & Med. **55**:165, 1944.

CORRELATION OF ROENTGENOGRAPHIC AND PATHOLOGIC OBSERVATIONS

The normal liver was visualized roentgenographically as a diffuse shadow. Within the first two months of the butter yellow–rice–carrot diet the roentgenograms of some animals showed a patchy concentration of thorium dioxide and the appearance of poorly defined "ring shadows." After two months of the diet the roentgenograms of a few of the rats showed fairly well defined ring shadows with thick walls alternating with areas of diffuse shadow. From the third month on, the ring shadows became more numerous and entirely replaced the diffuse shadow. The ring shadows were for the most part uniform in size. In some areas they were fairly large.

TABLE 1.—*Correlation of Gross and Microscopic Changes in the Liver with Roentgenographic Appearances After Variable Periods of Butter Yellow–Rice–Carrot Diet*

Rats	Duration of Diet	Pathologic Changes	Roentgenographic Appearance
2	2 wk.	Fatty metamorphosis	No changes
5	5 wk.	Diffuse cellular degenerative changes	No changes
5	2 mo.	Degenerative cellular changes to moderate fibrosis	No change in some; appearance of concentration of thorium dioxide and poorly defined ring shadows—cirrhotic configuration—in others
3	3 mo.	Degenerative cellular changes to moderate fibrosis	Distinct ring shadows—slight to moderate cirrhotic configuration
5	4 mo.	Moderate to considerable cirrhosis	Moderate to marked cirrhotic configuration
6	5 to 7 mo.	No cirrhosis in 1 rat; slight to marked cirrhosis in others; new growths in some animals	No cirrhosis in 1 rat; slight to marked cirrhotic configuration in others; new growth outlined in some animals

Histologic studies of liver tissue in an early stage of cirrhosis and prior to the appearance of connective tissue showed thorium dioxide within vessels of portal spaces and in sinusoidal capillaries. The homogeneous shadow of the roentgenogram was due to the diffuse spread of thorium dioxide in the sinusoidal capillary system. A part of the thorium dioxide was phagocytosed by large mononuclear cells, and the remainder was free. In those livers with considerable deposition of connective tissue, representing advanced cirrhosis, thorium dioxide became localized to the vessels of the perilobular and intralobular connective tissue. Thorium dioxide was present also in relatively large quantities in arteries of the portal spaces. There was little if any of the material in the sinusoidal capillaries of hepatic nodules surrounded by scar tissue. The ring shadows apparently represented connective tissue with concentration of thorium dioxide. The clear areas were the circumscribed

nodules of liver. Degenerative changes of the cells did not alter the roentgenographic shadow.

With advanced cirrhosis, evidenced by extensive and diffuse nodularity, the ring shadows occupied the entire liver. The degree and the extent of cirrhosis could be determined on the basis of the size, the number and the distribution of the ring shadows in the lobes of the liver (fig. 1).

By neither of the criteria, roentgenographic shadow or pathologic change, was there any uniformity of development or of degree of cirrhosis. Although all the animals were given the diet at the same time and were maintained in the same environment of controlled temperature and humidity, there was considerable variation in the appearance of the livers. Some of the rats failed to show cirrhosis after four to six months of butter yellow diet. Other animals showed extensive cirrhosis after two months of the diet. There was also considerable difference in various areas of the same liver. Some parts of the liver had extensive perilobular and intralobular fibrosis, with or without cellular degeneration, while in other areas the tissue appeared normal and without structural distortion. It was possible to identify all the degenerative and proliferative changes of cirrhosis in the same liver (fig. 2).

EFFECT OF RETURN TO NORMAL DIET ON CIRRHOTIC LIVERS

In six weeks after the animals were returned to their normal diet, roentgenographic changes became apparent in most of the animals. The shadow rings became larger and the walls thinner. In some areas the shadow rings became either indistinct or disappeared entirely. Histologically there was a reduction of connective tissue. Thorium dioxide was present in small quantities in the remaining connective tissue. It appeared to be concentrated in the vessels of the portal spaces.

In the subsequent periods up to the fifth month the hepatic changes were progressive but not significant. The shadows tended to become diffuse. In patchy areas the rings were reduced in number and increased in size. After the fifth month some of the animals showed complete return to a normal roentgenographic picture. In other rats the improvement varied from slight to moderate. New growths appeared in the livers of most of the animals. Appearance of and degree of recovery were not consistent in the animals. There was considerable variation among the livers of the rats as well as within the liver of the same animal. Histologic examination of one or two areas of a liver did not give a true picture of the whole organ. Gross study of slabs of liver with microscopic correlations of multiple grossly differing areas gave a truer evaluation (table 2 and fig. 3).

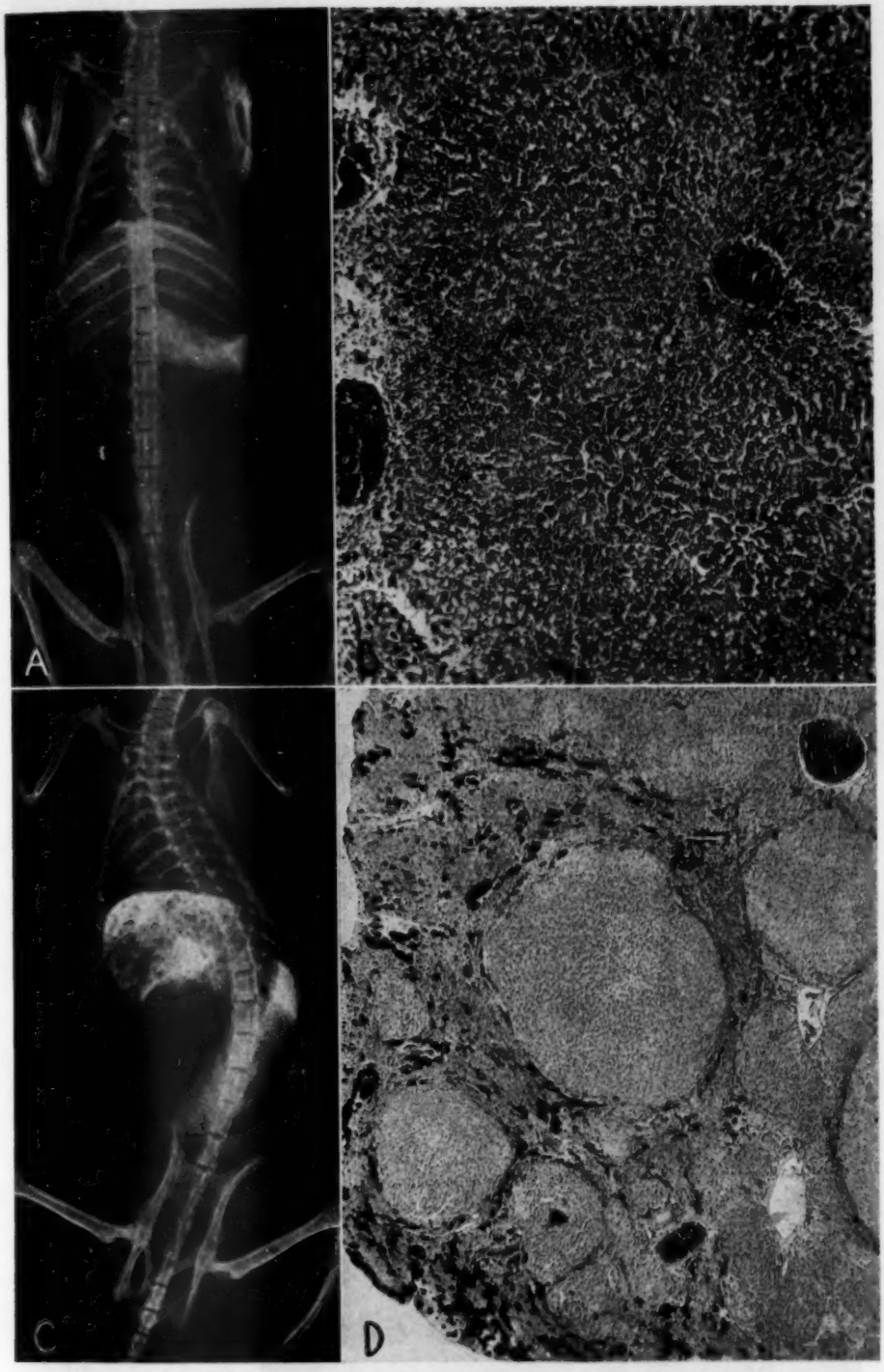

Fig. 1.—Correlation of roentgenographic and histologic changes of livers of rats fed a butter yellow–rice–carrot diet.

A, roentgenogram of a rat which had been maintained on the butter yellow–rice–carrot diet for one month and in which a colloidal suspension of thorium dioxide was then injected intracardially. The liver and the spleen are visualized as diffuse shadows, a roentgenographic appearance suggestive of normal organs.

B, section of the liver of the rat in *A*. Note the diffuse presence of thorium dioxide in the sinusoidal capillaries and in the vessels of the portal spaces. There are degenerative and congestive changes but no fibrosis. The diffuse distribution of the thorium dioxide is responsible for the homogeneous shadows.

C, roentgenogram of a rat fed the butter yellow–rice–carrot diet for two months, with thorium dioxide showing cirrhotic configuration. Note the ring shadows due to thorium dioxide present in the connective tissue.

D, section of the liver of the rat in *B*. Note the localization of thorium dioxide in the connective tissue around nodules of liver tissue which results in ring shadows.

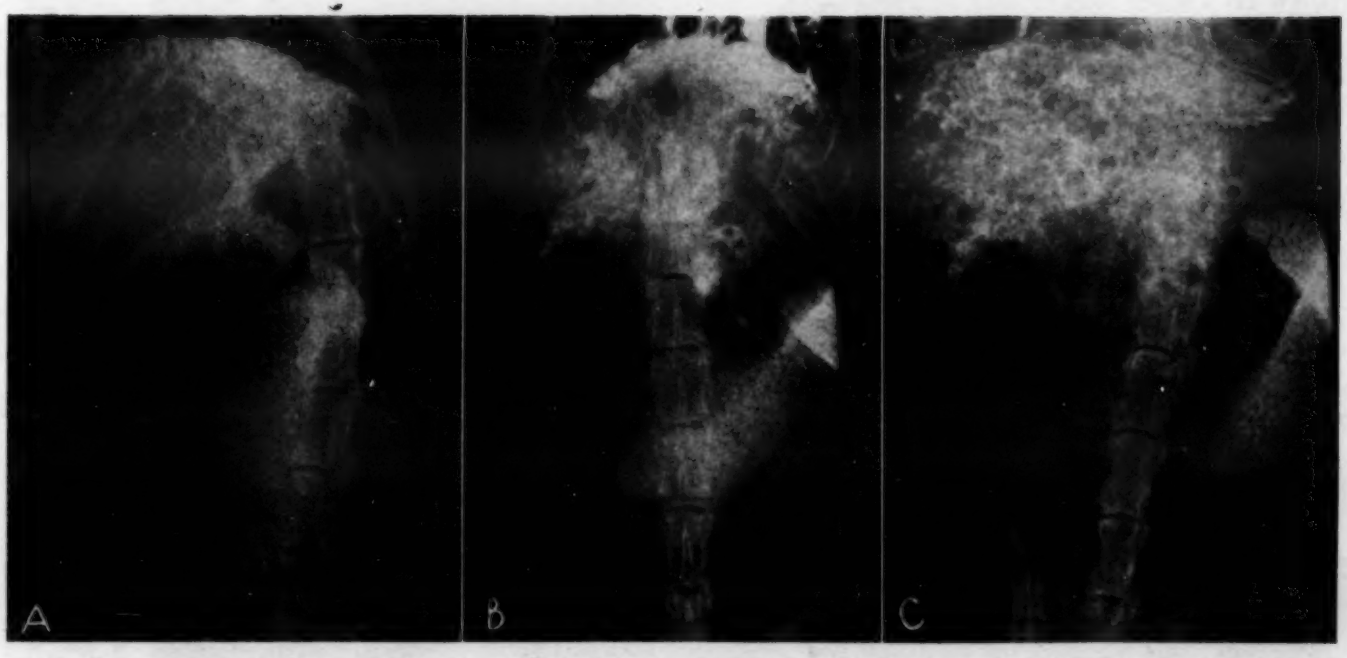

Fig. 2.—Roentgenograms of 3 rats which had been fed a butter yellow–rice–carrot diet for four months and in which a colloidal suspension of thorium dioxide was then injected intracardially. These roentgenograms show variable degrees of hepatic cirrhosis after the same period of butter yellow diet.

A, diffuse shadow indicating that no scarring has occurred. The roentgenogram is similar to that of a normal animal.

B, indistinct but fairly large ring shadows without clearcut walls, localized to a few areas. There is concentration of thorium dioxide in some areas. Persistence of diffuse shadow indicates an early stage of cirrhosis, with a small amount of connective tissue.

C, extensive presence of small ring shadows with fairly thick walls throughout the liver. This appearance is indicative of a fairly advanced diffuse cirrhosis.

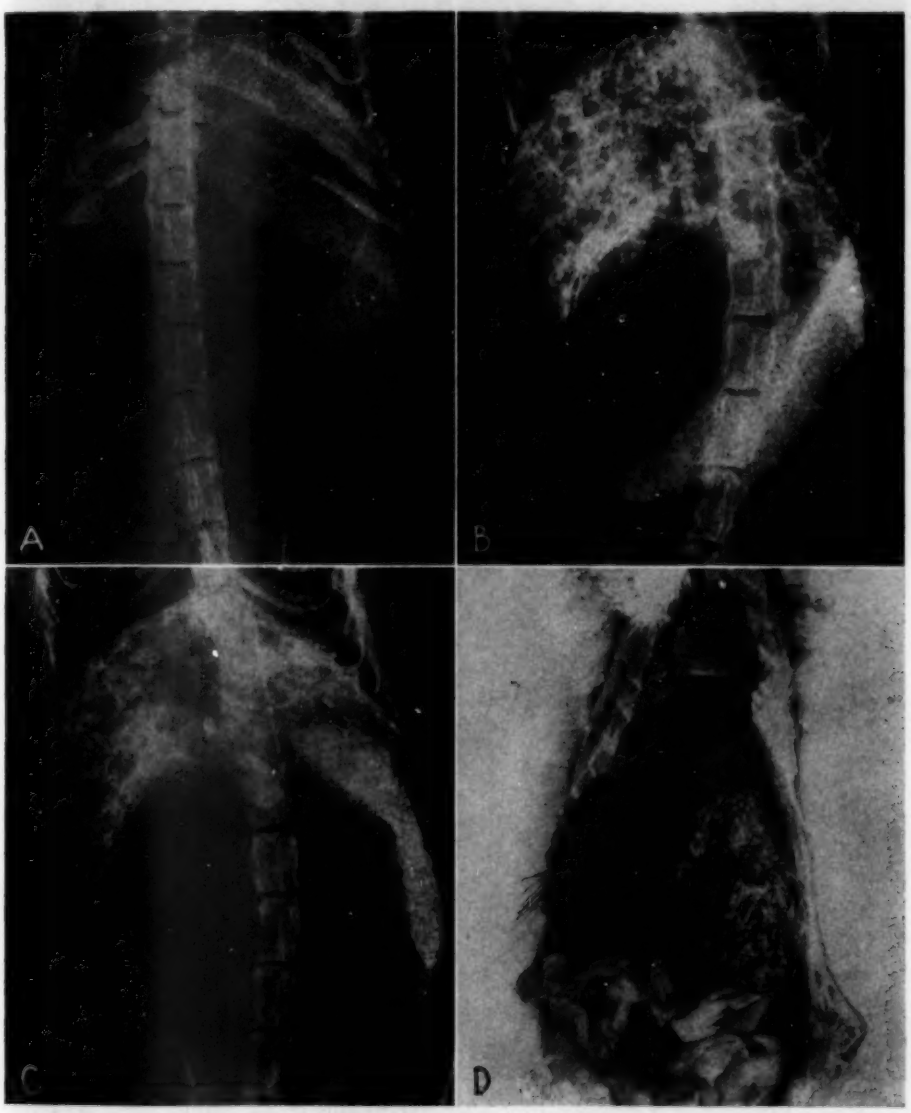

Fig. 3.—Roentgenograms and gross appearance of a rat fed butter yellow for four months and the normal stock ration for six weeks. *A*, roentgenogram before feeding of butter yellow. *B*, roentgenogram after four months of butter yellow–rice–carrot diet. Note the diffuse ring shadows. *C*, roentgenogram six weeks after return to the normal stock ration. Note the tendency to return to a diffuse shadow, the increase in the size of the ring shadows and the hazy ring walls.

D, gross photograph of the liver of the animal shown in *A*, *B* and *C*, taken six weeks after return to the normal diet. Note persistence of nodularity. Histologic sections showed extensive areas of structurally normal tissue and a decrease in scarring.

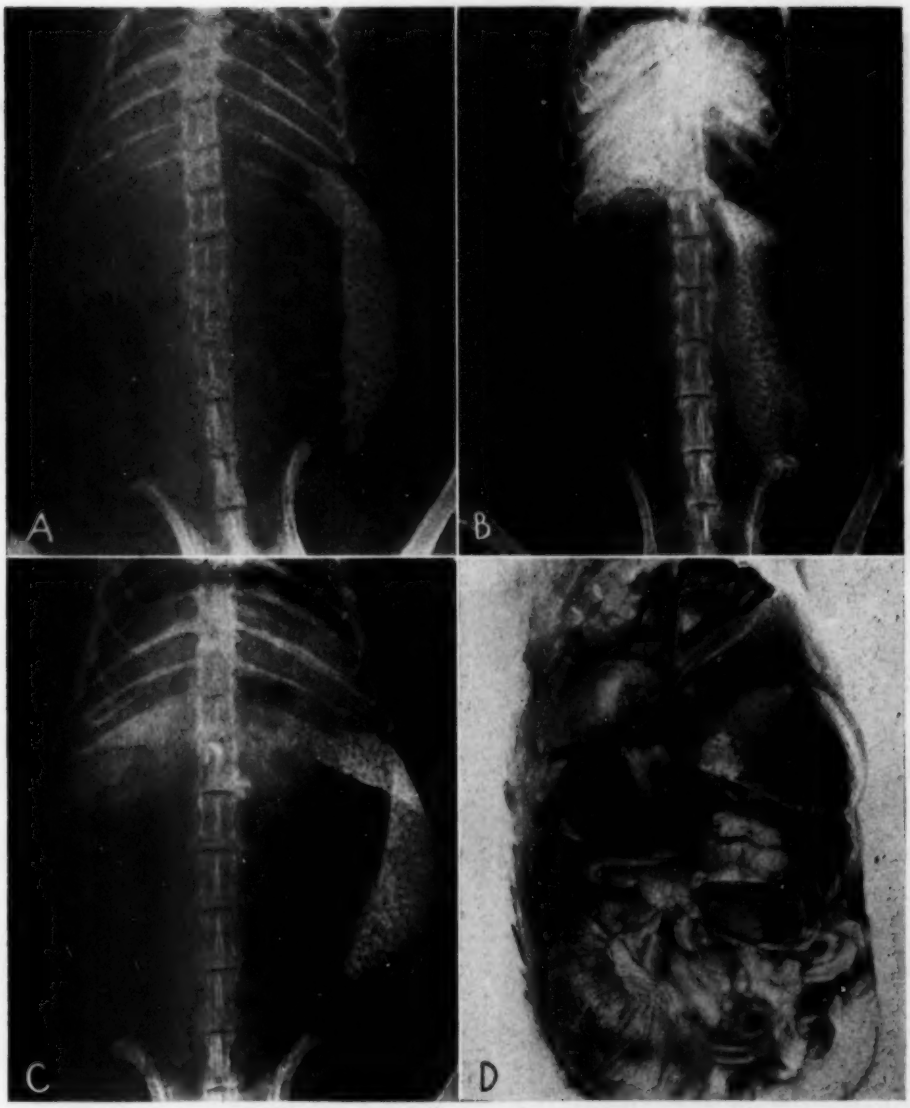

Fig. 4.—Recovery of a cirrhotic liver after five months of the normal diet. *A*, roentgenogram before feeding of butter yellow. *B*, roentgenogram after four months of the diet containing butter yellow. Note the configuration of moderate cirrhosis in small ring shadows. *C*, roentgenogram five months after return to the normal diet. Note the return to a diffuse shadow and the absence of ring shadows, indicating absorption of connective tissue.

D, gross photograph of the liver five months after return to the normal diet. Note absence of nodules and smooth surface, also one area of cancer.

After the fifth month the structure of the liver in some animals appeared normal. Little of the connective tissue which had subdivided the parenchyma remained. There were, however, multiple foci of cell degeneration, bile duct proliferation and new growth. Parts of livers had retained the nodularity and the connective tissue.

Increase in size of ring shadows on roentgenograms corresponded to reduction of or disappearance of connective tissue with presence of large areas of structurally normal liver. Thin walls of ring shadows indicated reduction in connective tissue. Thorium dioxide reappeared in small quantities in the sinusoidal capillaries. Absence of thorium dioxide shadows was associated with new growth. After the appearance

TABLE 2.—*Correlation of Pathologic Changes with Roentgenographic Appearances of Cirrhotic Livers After Return of Animals to a Normal Diet*

Rats	Duration of Butter Yellow-Rice-Carrot Diet, Months	Duration of Normal Diet	Roentgenographic Appearance	Pathologic Changes
2	4	2 wk.	No changes	No changes
4	4	6 wk.	Increase in size of shadow ring; decrease in ring walls	Decrease in connective tissue; larger structurally normal liver area
4	4–5	2 mo.	Progressive improvement up to 5th month, with reduction in number of ring shadows and appearance of patchy diffuse areas	Progressive improvement up to the 5th month, with reduction of scar tissue and return of structurally normal tissue
4	4–5	3 mo.		
2	4	4 mo.		
4	4	5 mo.	In some animals livers became normal; in others there was slight to marked improvement, with disappearance of ring shadows and return of diffuse areas of thinner than normal density	Return to normal in some livers; slight to marked improvement in others; disappearance of most of scar tissue in some rats; degenerative, hyperplastic and new growth changes
2	4	6 mo.		
2	3	8½ mo.		

of new growth intracardial injections of the colloidal suspension of thorium dioxide failed to produce visualization. Apparently the thorium dioxide did not find its way into the blood vessels of new growths (fig. 4).

SUMMARY

Nodular cirrhosis of the liver was produced in albino rats by a diet of butter yellow, rice and carrot. A colloidal suspension of thorium dioxide injected intracardially visualized the liver of the normal animal as a diffuse roentgenographic shadow. As cirrhosis developed the roentgenographic shadow was altered. Ring shadows replaced the normal diffuse appearance. These ring shadows indicated the degree and the extent of the cirrhosis.

On return of animals to a normal stock diet, some of them showed progressive improvement, with partial or complete disappearance of connective tissue and nodular cirrhosis. The improvement was char-

acterized by gradual replacement of ring shadows by diffuse shadows. The gross and histologic changes were found to correspond with the roentgenographic alterations.

CONCLUSIONS

Connective tissue which is laid down in nodular cirrhosis induced by a butter yellow diet is reabsorbed in part or completely after the animals have been returned to a normal stock diet. There is also partial or complete restoration of the structural integrity of the hepatic parenchyma. The improvement becomes apparent within six weeks after the animal has returned to a normal diet. An approximate period of five months is required for significant absorption of connective tissue.

The progressive changes in nodular cirrhosis of the liver of the rat and the gradual return of the normal structure can be delineated roentgenographically by injecting intracardially a colloidal suspension of thorium dioxide.

The development of nodular cirrhosis and the return to normal are neither uniform in all animals nor uniform in the liver of the same animal.

MICROSCOPIC LESIONS IN ACETYLCHOLINE SHOCK

RUDOLF ALTSCHUL, M.U.Dr., and M. M. LASKIN, B.A.
SASKATOON, SASKATCHEWAN, CANADA

THE animal experiments reported here were undertaken for the purpose of ascertaining whether the administration of very large, and even lethal, doses of acetylcholine and neostigmine would produce definite microscopic changes of tissue. If such changes occurred, it should be decided whether they were of a nature to permit them to be classified as "shock," though, admittedly, microscopic changes in shock are not, as a rule, definitely characteristic.

From an analysis of its neurogenic effects, shock has been considered to be the result of an overstimulation of the sympathetic nervous system or, what is probably the same, an overaction of epinephrine. On the other hand, shock has also been regarded as due to hyperfunctioning of the parasympathetic system and thus as being possibly a sequel to strong parasympathicomimetic drug action.

The symptoms caused by strong doses of acetylcholine resemble so closely those in anaphylactic shock that "acetylcholine shock" has by some authors been considered to be closely related to anaphylactic shock if not actually identical with it (Nakamura,[1] Danielopolu[2]).

Danielopolu[2] suggested the name "paraphylactic shock" because of the absence of sensitization in acetylcholine shock. For a similar reason, the reaction to acetylcholine may be classified, according to Wells,[3] as anaphylactoid shock. (See also Moon[4] and Wiggers.[5])

In recent years acetylcholine has been used to produce shock as a therapeutic agent in certain mental diseases, but the results so far have not been especially encouraging (Cohen, Thale and Tissenbaum;[6] Harris and Pacella[7]).

From the Department of Anatomy, University of Saskatchewan.

This investigation was aided by a grant from the National Research Council of Canada.

Most of the drugs used in the experiments reported in this paper were supplied by Mr. Paul Blanc (Hoffmann-LaRoche Ltd., Montreal, Canada).

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(Footnotes continued on next page)

Farber, Pope and Landsteiner [8] observed that in only 10 per cent of the animals which had died from anaphylactic shock did the hearts show an increased acetylcholine content; in the other organs no increase was found.

Frommel, Thalheimer, Herschberg and Piquet [9] explained that the vagotonia in traumatic shock is caused by functional inhibition of cholinesterase action, allowing an increase of acetylcholine effect. The treatment of shock with cholinesterase (Schachter [10]) corresponds to this view.

In an earlier publication [11] one of us (R. A.) approached the problem of cholinergic shock in an experimental way, but the organs of the animals which had died after being injected with large doses of parasympathicomimetic drugs were not systematically examined.

MATERIALS AND METHODS

In the present study 24 white rats, 16 guinea pigs and 12 rabbits were shocked by intramuscular or intraperitoneal injections of strong lethal or sublethal doses of acetylcholine chloride, neostigmine methylsulfate or epinephrine hydrochloride. The doses were chosen and arranged so as to allow for varying intervals between the time of injection and the death of the animal. In several cases the doses were repeated at relatively short intervals, the animal dying after the last injection. The smallest single dose of acetylcholine chloride was 0.04 Gm. per thousand grams of body weight; the largest single doses, 0.42 Gm. per thousand grams of body weight. The smallest single dose of neostigmine methylsulfate was 0.2 cc. and the largest 3 cc. of a 1:2,000 solution per thousand grams of body weight. The shortest interval between injection and death was one minute; the longest between the first injection and death was one hundred and twenty-two minutes. Longer intervals (five hours) were noted with epinephrine. Moreover, numerous other animals were used for ascertaining threshold doses of the substances.

The lungs of the animals after death were fixed in situ by careful intratracheal instillation of Heidenhain's Susa fixative or of alcohol-solution of formaldehyde B.P. or solution of formaldehyde B.P. In a few instances the lungs were cut out after the trachea had been firmly ligated to prevent collapse of the lungs. But even in these specimens some injection fluid was gently instilled intratracheally so as to permit partial fixation from within.

The abdominal organs were first fixed in situ; after a few hours they were cut out, trimmed into blocks and refixed.

Various histologic stains were used: Hematoxylin-eosin, acid hematoxylin of Mallory, which stains fibrin well, and Mallory's connective tissue stain, which

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10. Schachter, R. J.: Am. J. Physiol. **143**:552, 1945.

11. Altschul, R.: J. Nerv. & Ment. Dis. **99**:895, 1944.

shows the filling stage of blood vessels; further, iron and iron pigment stains and the Foot-Hortega reticulin stain were used.

The lungs, the heart, the liver, the kidneys, the spleen and the large and the small intestine were regularly searched. In a few cases the brain and the adrenal glands were also examined microscopically.

RESULTS

The results may be grouped according to the animal species and according to the drug used (viz., acetylcholine, neostigmine or, for comparative reasons, epinephrine). No pathologic changes were observed in the intestines, and therefore these will not be described in the following report. Similarly, the findings in the brain and in the adrenal glands will be omitted, since these organs were not searched regularly.

White Rats.—(*a*) Acetylcholine and Neostigmine: The ventricles of the heart were found either in systole or in diastole, with no relation to the drug used, sometimes the left being in systole and the right in diastole. The myocardium showed capillary and venous hyperemia. In several specimens the arterioles were strongly contracted.

The kidneys revealed prevalently venous and capillary hyperemia, the liver prevalently venous and sinal hyperemia and the spleen moderate or no hyperemia.

In the majority of the rats there was capillary and venous hyperemia of the lungs. Intra-alveolar hemorrhages were observed in a few. Of 7 animals given intramuscular injections of acetylcholine chloride, 3 showed edema, 1 questionable edema and 3 none. No edema was found in another animal which had received acetylcholine chloride intraperitoneally. Of 5 animals shocked with intramuscular injections of neostigmine methylsulfate, 3 showed edema of the lungs and 2 did not. Of 5 rats treated with intraperitoneal injections of neostigmine methylsulfate, 1 showed a very slight degree of edema, while 4 showed no edema.

(*b*) Epinephrine: Seven rats were given injections of epinephrine hydrochloride for control purposes. Four which had received the drug intramuscularly showed intensive pulmonary edema, while of 3, in which the drug had been injected intraperitoneally, 1 showed no pulmonary edema and 2 showed very slight or doubtful edema of the lungs.

Guinea Pigs.—(*a*) Acetylcholine and Neostigmine: As in the rats, the ventricles of the heart were found in systole or in diastole, with no relation to the drug used. The veins and the capillaries were hyperemic in the majority of the animals, but the arterioles showed no definite contraction.

The kidneys generally showed venous hyperemia, mostly cortical. In half of the animals the arterioles were strongly contracted.

Various degrees of sinal and venous hyperemia were observed in the liver. The vasodilatation was more accentuated in those guinea pigs in which the drug had been injected intraperitoneally. In 3 animals the arterioles were extremely contracted. In 2, necrotic areas of the parenchyma were found, which probably were not connected with the state of shock, since the latter lasted in these animals eleven minutes and one hundred and six minutes, respectively. Two specimens appeared to be completely normal.

In the spleen all degrees of sinal and venous hyperemia were noted, but in some specimens the vessels appeared normal. In 1 animal, which had been given acetylcholine chloride intramuscularly and in 1 which had received neostigmine methylsulfate, also intramuscularly, the spleen appeared very "juicy" (edematous).

Of 8 animals given acetylcholine chloride intramuscularly, 3 showed pulmonary edema; 3 did not. In 1 the edema, if present, was of such little intensity that it may be classified as questionable. The lungs of 1 animal had to be discarded since they presented signs of incipient pneumonia, which obscured the investigation. Four animals received neostigmine; of 3 in which the drug was intramuscularly injected, 2 reacted with strong edema of the lungs, while the third showed only a very slight, questionable edematous reaction. Two animals in which the drug was intraperitoneally injected also showed very slight, even questionable pulmonary edema.

(*b*) Epinephrine: Epinephrine hydrochloride injected intramuscularly in 3 animals caused strong edema, but when injected intraperitoneally it caused in 1 animal a distinctly lesser pulmonary edema and no edema in 1 other.

Rabbits.—(*a*) Acetylcholine and Neostigmine: Systolic and/or diastolic standstill of the cardiac ventricles occurred without relation to the drug used, to the way of its administration or to the duration of the shock. There was moderate or no hyperemia of the myocardial capillaries. In the majority of the specimens venous hyperemia was noted; the arteries and the arterioles were only slightly contracted or not contracted at all.

The liver showed various degrees of sinal and venous hyperemia, ranging from strong to slight. In some specimens hyperemia was lacking. The arteries and the arterioles appeared to be normal.

In the majority of the rabbits the sinuses and the veins of the spleen were strongly hyperemic. In 2 animals the organ was distinctly edematous.

Acetylcholine chloride intramuscularly injected gave no edema in the lungs of 1 animal and slight edema in those of 1 other. Intraperitoneally applied in 1 animal, it caused no edema. Neostigmine methylsulfate, intramuscularly injected in 2 animals, did not provoke edema. Intraperitoneally injected it caused moderate edema in 2 animals but no edema in 2 others.

(*b*) Epinephrine: Epinephrine hydrochloride when given intramuscularly in 1 animal produced strong edema of the lungs, but when given intraperitoneally in another animal, provoked only moderate pulmonary edema.

COMMENT

Microscopic examination of various organs of animals which have been subjected to large doses of acetylcholine chloride or neostigmine methylsulfate shows great variation of the filling stage of blood vessels and frequent, but not regularly occurring, pulmonary and splenic edema. These changes correspond to the anatomic picture of shock.

As is well known, epinephrine also causes pulmonary edema. Thus we are confronted with the fact that antagonistic substances—two parasympathicomimetic and one sympathicomimetic drug—elicit the same change, i. e., pulmonary edema.

However, further study may reveal that there are different mechanisms producing edema in the cases of epinephrine-induced edema and of cholinergic edema.

The vascular changes and the pulmonary edema observed in white rats and guinea pigs were much more typical of shock than were the corresponding findings in rabbits. On the other hand, the epinephrine-

induced pulmonary edema in the rabbit was incomparably stronger than the cholinergic edema in the few cases in which this was produced. This is not surprising in view of the species-bound differences of cholinergic sensitivity (Altschul [11]) and also in view of the fact that, as Best and Taylor [12] have pointed out, the pulmonary vessels of the rabbit react with constriction to vagal stimulation, whereas in other animals vasodilatation occurs. But a review of this problem by de Burgh Daly [13] makes one doubt any final statements regarding the vegetative innervation of pulmonary vessels.

There are in the literature some other examples of apparently synergic action as well as of apparently paradoxic action of sympathicomimetic and parasympathicomimetic substances. Grollman [14] stated that "epinephrine exerts certain effects which indicate a parasympathetic action;" Danielopolu [2] ascribed to acetylcholine an amphomimetic action. Exophthalmos is usually considered to be due to sympathetic reaction, but Brunton [15] described exophalmos produced in dogs with acetylcholine. In my own experience the exophthalmos produced in rats by rather strong doses of neostigmine surpasses even the exophthalmos induced with acetylcholine. It is possible that the mechanisms of the sympathicogenic and the cholinergic exophthalmos are different, but the ultimate explanation of their nature has yet to be furnished.

Besides this, attention may be directed to the observations that acetylcholine and epinephrine exert identical action on the nictitating membrane (Morrison and Acheson [16]), that the sympathetic cerebral centers are excited by acetylcholine after removal of the frontal lobes (Stavraky [17]) and that epinephrine and neostigmine show mutual reenforcement in the guinea pig (Altschul [11]). Luco [18] found that "adrenaline increases the responses of denervated muscles to acetylcholine."

Thus the occurrence of either an acetylcholine shock or an epinephrine shock does not necessarily exclude the occurrence of the other; pulmonary edema caused by parasympathicomimetic substances will have to be accepted as well as that brought about by sympathicomimetic substances.

It is conceivable that the apparently paradoxic actions of acetylcholine and neostigmine are due to cholinergic mobilization of a large

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17. Stavraky, G. W.: Tr. Roy. Soc. Canada (Sect. 5) **37**:127, 1934.

18. Luco, J. V.: Am. J. Physiol. **125**:196, 1939.

amount of epinephrine in the animal. A new series of experiments with scope surpassing that of the present work will have to be carried out on adrenalectomized animals in order to clarify the question of the ultimate mechanism in cholinergic shock and also of the other paradoxic phenomena of cholinergic action, referred to in the foregoing paragraphs.

The fact that acetylcholine and neostigmine, as well as epinephrine, acted much more strongly when administered intramuscularly than when given intraperitoneally deserves attention and perhaps further investigation. The slowing down of the action of epinephrine and of that of parasympathicomimetic drugs, as well, and the consequent increase of their threshold may be caused by different mechanisms. A tentative explanation is that in the first case the delay may be due to constriction of the peritoneal vessels, while in the second case it may be due to a diluting and delaying action by the blood reserve in the liver. Other factors may play minor or major roles.

SUMMARY

Sublethal and lethal doses of acetylcholine chloride, neostigmine methylsulfate and, for control purposes, epinephrine hydrochloride were injected into white rats, guinea pigs and rabbits.

Microscopic examination of various organs of the test animals showed a vascular reaction in many of them which resembled that described in other types of shock.

Moreover, in about half of the rats and the guinea pigs, the cholinergic drugs caused pulmonary edema. This supports still more the view that cholinergic shock may be accepted and correlated with other shock reactions.

The findings in the rabbits were less convincing, especially the reactions of the pulmonary vessels. This fact is not surprising since the pulmonary vessels of the rabbit give a constrictor response to vagal stimulation.

Attention is drawn to the fact that both epinephrine and cholinergic substances reacted much more slowly and weakly when they were applied intraperitoneally than when they were given intramuscularly.

The sympathetic and the parasympathetic stimulation produced several synergic and even paradoxic effects.

RELATION OF CHOLELITHIASIS TO ACUTE HEMORRHAGIC PANCREATITIS

D. W. MOLANDER, B.S., and E. T. BELL, M.D.
MINNEAPOLIS

IN 1901 Opie [1] reported a case of acute pancreatitis associated with a gallstone impacted in the ampulla of Vater. He suggested that a gallstone may produce pancreatitis in this way by diverting bile into the pancreatic duct. Cases of the type described by Opie are seldom seen at necropsies, but many pathologists have the impression that there is a high incidence of disease of the gallbladder in association with acute pancreatitis. However, no statistical evidence has been presented. Rich and Duff [2] maintained, on the contrary, that disease of the gallbladder is not unusually frequent in patients with acute pancreatitis; they attributed the pancreatic lesion to obstruction of the intrapancreatic ducts as a result of hyperplasia of the epithelial lining of these ducts.

The autopsy records of the department of pathology of the University of Minnesota show acute hemorrhagic pancreatitis encountered in 158 of 41,333 persons (26,262 males, 15,401 females) examined post mortem. Ninety-three of the patients were males, and 67 were females, but since there were about twice as many males as females among persons over 30 years of age coming to necropsy, the true proportion is about 10 males to 17 females.

TABLE 1.—*The Incidence of Cholelithiasis in Association with Acute Pancreatitis*

Decade of Life	Persons Showing Acute Pancreatitis		Persons Showing Acute Pancreatitis Associated with Cholelithiasis		Persons Showing Cholelithiasis at Necropsy According to Ludlow's Report	
	Males	Females	Males, %	Females, %	Males, %	Females, %
1	3	3	0	0	0.0	0.0
2	0	2	0	0	0.88	1.54
3	2	1	0	0	2.52	4.92
4	14	8	28.6	37.5	2.40	7.39
5	12	20	25.0	85.0	6.30	16.66
6	25	14	32.0	85.7	10.66	22.62
7	22	10	50.0	90.0	15.09	24.76
8	12	7	50.0	57.1	13.73	26.83
9	3	2	33.3	50.0	0.0	50.0
Total	93	67	36.5	68.7	5.77	10.45

In the accompanying table the frequency of cholelithiasis associated with acute pancreatitis in the aforementioned necropsy series is shown

From the Department of Pathology, University of Minnesota.

1. Opie, E. L.: Bull. Johns Hopkins Hosp. **12**:182, 1901.
2. Rich, A. R., and Duff, L. G.: Bull. Johns Hopkins Hosp. **58**:212, 1936.

along with Ludlow's [3] report of the occurrence of cholelithiasis in 4,800 persons (2,952 males, 1,848 females) examined post mortem. In each decade of life the number of cases of pancreatitis is too small to give a significant percentage, but the total group seems sufficiently large to justify the conclusion that cholelithiasis is observed much oftener in necropsies on persons with acute pancreatitis than in autopsies on people in general. It is clear that in about two thirds of the males and one third of the females with acute pancreatitis this disease develops in the absence of gallstones, but since gallstones are found about six times as frequently in both males and females with acute pancreatitis as in the necropsy population in general there must be some causal relation between the two findings. The mechanism by which gallstones produce pancreatitis has not been fully explained, but there is some evidence for the older view that they produce temporary obstruction of the papilla of Vater and divert bile into the pancreatic ducts.

3. Ludlow, A. I.: Am. J. M. Sc. **193**:481, 1937.

EXPERIMENTAL PITUITARY DIABETES OF FIVE YEARS' DURATION WITH GLOMERULOSCLEROSIS

F. D. W. LUKENS, M.D.
AND
F. C. DOHAN, M.D.
PHILADELPHIA

FOLLOWING the discovery of insulin and the finding that raw pancreas, as well as insulin, was required for the extended survival of depancreatized dogs, several investigators observed diabetic dogs for long periods. As no comparable reports on pituitary diabetes have appeared, this account of a dog with that type of diabetes, which was studied for five years, is presented. In table 1 the literature on experimental diabetes of long duration has been collected. According to this table, the case presented in the following pages is the fourth case of experimental diabetes and the first of pituitary diabetes of five years' duration to be recorded.

The diabetes produced in this dog with a crude saline extract of carefully dissected anterior lobes of beef pituitary glands [1] and some of the metabolic studies have been described in previous reports. The animal was first listed as dog I [2a] and later as dog P 16.[2b,c] Metabolic studies subsequent to those reports have shown no important changes, so that this type of data need not be repeated. The persistence and the constant degree of severity of the diabetes are shown in table 2, which requires brief comment. The period of the injection of the pituitary extract has been excluded, and data obtained during any kind of treatment have been omitted from table 2. The blood chemical values are averages of five to twenty determinations except in the instance of the serum lipids, which were measured only twice in the fifth year. The proportion of the available carbohydrate of the diet excreted in the urine is used as an approximate measure of the severity of the disease. (It is calculated from a dextrose-nitrogen

From the George S. Cox Medical Research Institute, University of Pennsylvania.

1. The preparation of the extract has been described elsewhere (Young, F. G.: Biochem. J. **32**:513, 1938).

2. (*a*) Dohan, F. C., and Lukens, F. D. W.: Am. J. Physiol. **125**:188, 1939. (*b*) Dohan, F. C.; Fish, C. A., and Lukens, F. D. W.: Endocrinology **28**:341 (March) 1941. (*c*) Dohan, F. C.; Chambers, A. H., and Fish, C. A.: ibid. **28**:566, 1941.

TABLE 1.—*Summarized Review of Literature on Prolonged Experimental Diabetes*

Reference	Species (Animals)	Duration of Diabetes	Lesions
Diabetes Produced by Total Pancreatectomy			
Hedon, E.: Compt. rend. Soc. de biol. **100**: 698, 1929	Dog (1)	5 years	Renal damage
Macleod, J. J. R.: Carbohydrate Metabolism and Insulin, London, Longmans Green & Co., 1926, p. 88	Dog (2)	4 years	Fatty liver, aortic lesions
Chaikoff, I. L., and Kaplan, A.: J. Nutrition **14**: 450, 1937	Dog (4)	2 for 4 years 2 for 5 years	Cataracts, lipemia, fatty liver
Dragstedt, L. R.; Goodpasture, W. C.; Vermeulen, C., and Clark, D. E.: Am. J. Physiol. **126**: 479, 1939	Dog (6)	Not stated	Arteriosclerosis
Diabetes Produced by Partial Pancreatectomy			
Sandmeyer, W.: Ztschr. f. Biol. **31**: 12, 1895	Dog (2)	2 and 8 months	Cachexia only
Langfelt [3]	Dog (4)	9, 12, 13 and 20 months	Hyperplasia of thyroid gland
Fisher, N. F.: Am. J. Physiol. **67**: 634, 1923	Dog (1)	8 months	Arteriosclerosis
Diabetes Produced by Injections of Pituitary Extract			
Richardson, K. C., and Young, F. G.: Lancet **1**: 1008, 1938. Richardson [4]	Dog (5)	25 to 58 weeks	Pancreatic lesions only
Ham, A. W., and Haist, R. E.: Am. J. Path. **17**: 787, 1941	Dog (3)	78 to 198 days	Pancreatic lesions only
Dohan and others [5b]	Dog (6)	92 to 418 days	Pancreatic lesions only
This report	Dog (1)	5 years	Pancreatic and renal lesions
Lukens, F. D. W., and Dohan, F. C.: Endocrinology **30**: 175, 1942. Lukens F. D. W.; Dohan, F. C., and Wolcott, M. W.: ibid. **32**: 475, 1943	Cat (14)	142 to 463 days	Pancreatic lesions only
Diabetes Produced with Alloxan			
Bailey, O. C.; Bailey, O. T., and Leech, R. S.: New England J. Med. **230**: 533, 1944	Rabbit	Late findings	Pancreatic and renal lesions of alloxan
Goldner, M. G., and Gomori, G.: Proc. Am. Diabetes A. **4**: 80, 1944	Dog, rat	Unreported	Cataract

TABLE 2.—*Course of Diabetes of Dog P 15*

Year of Diabetes	Blood Sugar, Mg. per 100 Cc. (Average)	Serum: Fatty Acids, per Cent (Average)	Serum: Cholesterol, Mg. per 100 Cc. (Average)	Glycosuria, per Cent of Dietary Carbohydrate	Ketonuria	Time Treated per Year, Days
1	200	0.641	...	76	0 to 4+	53
2	198	0.552	365	87	0 to 4+	91
3	219	0.513	396	82	0 to 1+	153
4	194	.....	...	86	0 to 2+	0
5	217	0.860	210	84	0 to 1+	46

ratio of 3.65.) The average value for glycosuria is based on two week metabolic periods near the end of each year. In the period for the first year the diet was 1,000 Gm. of beef daily. In the other metabolic

periods it was 700 to 800 Gm. per day. The practically uniform diet during the later periods adds validity to this index of the constancy of the diabetes.

TREATMENT

The treatment of this animal included injections of insulin, reduction of the diet and one course of treatment with phlorhizin. In the first year the treatment consisted of numerous short periods of dietary restriction. The first course of insulin treatment was not begun until the five hundred and thirty-seventh day of diabetes (four hundred and ninety-four days after the last injection of the pituitary extract). Insulin was used for fifty-three days, and fairly good control of the diabetes was achieved, the morning blood sugar ranging from 63 to 177 mg. per hundred cubic centimeters during treatment. Glycosuria was reduced from an average of 61 Gm. to about 5 Gm. per day on the same diet. Short courses of insulin therapy were given in the third and fifth years, but insulin was used for a total of only one hundred and eighty-six days of the animal's five years of diabetes. The insulin requirement from year to year is shown in table 3:

TABLE 3.—*Insulin Requirement of Dog P 16*

Year	Meat, Gm. per Day	Insulin, Units per Day
2d	800	32
3d	700	30
5th	800	36

The failure to lessen the diabetes by treatment begun late in the disease (i. e., four to six weeks after the onset of glycosuria in dogs) has been described before.[2b] There was one thirty-four day period of a pure fat diet, during which the animal showed only traces of glycosuria, ketonuria (4 plus) and a blood sugar level of 169 to 175 mg. per hundred cubic centimeters. Otherwise, periods of dietary treatment were never longer than three to six day metabolic periods. They have been included to indicate the time during which the diabetes was temporarily altered by changes in regimen. It is clear that the total time of all types of treatment was a small fraction of the five years of diabetes, and that these types of treatment did not influence the course of the disease. The animal's weight was maintained during the long periods of maintenance diet without treatment.

For the two weeks preceding its death the dog was in excellent condition on a diet of 750 Gm. of meat daily and excreted an average of 68 Gm. of dextrose per day. Traces of acetone were present in the urine. The animal weighed 8.5 Kg. at the beginning of the experiment and 11.6 Kg. at autopsy five years later. Autopsy was

begun with the animal under pentobarbital sodium anesthesia in order that glycogen might be determined.

TERMINAL CHEMICAL DATA

Hepatic glycogen was evaluated as 1.14, cardiac glycogen as 0.4 and renal glycogen as 0.23 per cent. The blood sugar amounted to 242 mg., the blood urea nitrogen to 21 mg. and the serum cholesterol to 208 mg. per hundred cubic centimeters. The values for fatty acids were, serum 0.90, liver 22.0, and diaphragm 0.33, per cent.

NECROPSY

The pancreas weighed 30 Gm. and was grossly normal. The liver weighed 600 Gm. and was very fatty and friable. The two adrenal glands together weighed 1.56 Gm. and appeared normal. The kidneys were pale with yellowish striations. Other organs were grossly normal.

MICROSCOPIC EXAMINATION

No abnormalities were found in the following organs: heart, lungs, stomach, duodenum, spleen, adrenal glands, thyroid gland and parathyroid glands. The normal thyroid gland, with flat or cuboidal epithelium lining the acini, is noted in contrast to the gland showing hyperplasia reported by Langfelt.[3] The splenic and coronary blood vessels were unchanged. The eyes were found normal by Dr. Irving Leopold, of the department of ophthalmology of the University of Pensylvania.

In the pancreas the acinous tissue and the blood vessels were normal. There was slight vacuolation of the epithelium of the small ducts.[4] The islands of Langerhans were slightly fewer than normal, and all were small and irregular in shape (fig. 1). Bensley stains were not made, but even with hematoxylin and eosin it seemed that some beta cells were present. There was hydropic degeneration in an occasional beta cell. The Masson and Mallory stains revealed no hyaline change in the islands. The condition of the islands conformed to previous descriptions of the atrophy seen late in pituitary diabetes.[5]

The hepatic parenchyma was extensively and diffusely infiltrated with fat, and this was confirmed by fat staining. The ducts and the vessels were normal, and there was no cellular or inflammatory reaction and no excess of connective tissue.

The kidneys showed glomerular and tubular lesions. The glomerular lesions resembled the early changes of intercapillary glomerulosclerosis as described and reviewed by Allen.[6] All of the glomeruli

3. Langfelt, E.: Acta med. Scandinav. **53**:1, 1920.
4. Richardson, K. C.: Proc. Roy. Soc., London, s.B **128**:153, 1940.
5. Richardson.[4] Dohan and others.[2b]
6. Allen, A. C.: Arch. Path. **32**:33, 1941.

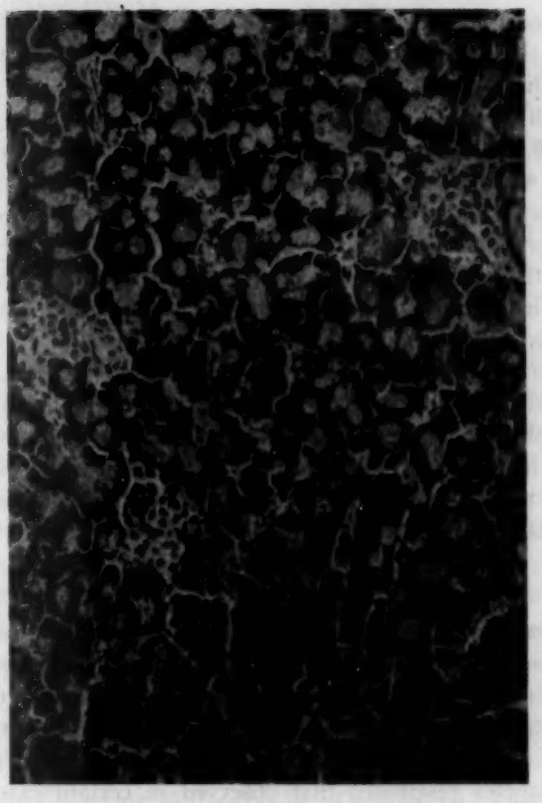

Fig. 1.—Pancreas; × 177.

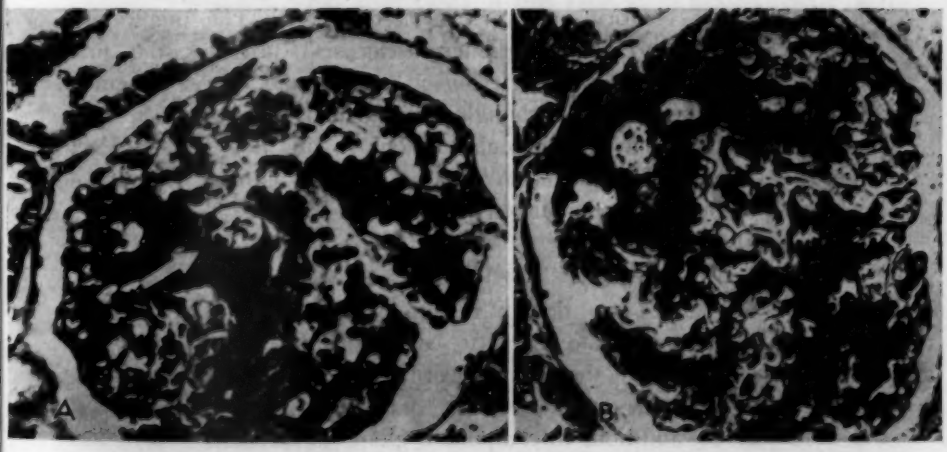

Fig. 2.—In *A* the thickened capillary wall surrounding a lumen containing erythrocytes is shown by the arrow. Another such capillary is seen beyond it in the direction of the arrow. Focal cellular proliferation of the type shown here was present in all glomeruli. Hematoxylin and eosin; high power magnification. In *B* the patchy deposition of hyalin in a glomerulus is shown by the Masson stain under high power magnification.

had small focal hyaline deposits in the walls of the capillaries. The hyalin took the form of a thin ring or crescent of eosinophilic material next to the lumen and was also seen as irregular deposits (fig. 2). In all glomeruli the hyaline change was overshadowed by the striking focal cellular proliferation. The cells, which appeared to be endothelial, were most conspicuous around the afferent arterioles. Occasional arterioles outside the glomeruli were also involved. There were no leukocytes or other evidence of inflammation. In his review Allen [6] noted that the cellularity of the lesions of glomerulosclerosis varied widely, and his illustrations suggest that the cellular collections are more prominent when the hyaline change is minimal or early. Hyaline thickening of Bowman's capsule was frequently observed.

The tubules were the site of extreme patchy fatty infiltration, which fat staining showed was accompanied by fine fatty deposition throughout the organ, including the glomeruli. Such fatty changes have been described in the kidneys of diabetic and some nondiabetic patients. The larger vessels and collecting tubules were not remarkable.

COMMENT

The observation that pituitary diabetes was present for five years after the cessation of injections of pituitary extract supports the use of the term "permanent" in describing this diabetes. After the first year of the condition, neither hyperglycemia nor any other factor increased the severity of the disease. The constancy of this dog's essentially untreated diabetes resembled that observed in certain cases of human diabetes. At autopsy the lesions were limited to those of diabetes. The atrophy of the islands of Langerhans and the fatty deposits in the liver and the kidneys conform to previous descriptions. The unexpected occurrence of "intercapillary glomerulosclerosis" provides the first example known to us of this lesion in an animal. It is generally regarded as a diabetic lesion, although this statement is not above controversy. The glomerular and vascular lesions in this dog were distinct from the lymphocytic infiltration and scarring of chronic interstitial nephritis which occurs spontaneously in dogs.[7]

SUMMARY

Diabetes produced in a dog by injections of a pituitary extract was observed for five years and found to be of constant severity after the first year. At autopsy the lesions were those which have been previously noted in cases of diabetes, viz., fatty deposits in the liver and the kidneys. In addition, intercapillary glomerulosclerosis, which has not hitherto been reported in experimental diabetes, was noted.

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7. Morehead, R. P., and Little, J. M.: Am. J. Path. **21**:339, 1945.

MORPHOLOGIC STUDIES OF RATS DEPRIVED OF ESSENTIAL AMINO ACIDS: III. HISTIDINE

MARK E. MAUN, M.D.; WILLIAM M. CAHILL, Ph.D.
AND
RUTH M. DAVIS, B.A.*
DETROIT

YOUNG rats fed synthetic diets devoid of a single essential amino acid survived an experimental period of one month with difficulty; during this period the rats lost weight, became very weak and lost their healthy appearance.[1] Examination of rats after they had consumed diets in which either phenylalanine or leucine was absent revealed marked thymic atrophy; atrophy and decrease of lipids of the adrenal cortex, degeneration and atrophy of the epithelium of the seminiferous tubules and thinning of the epiphyses of the long bones. In addition, leucine-deficient animals had striking ocular changes, similar to those previously reported by investigators [2] who studied tryptophane and lysine deficiencies. These changes consisted of thinning and metaplasia of the corneal epithelium with edema and leukocytic infiltration of the substantia propria.

In observations reported thus far by us reductions of hemoglobin and plasma protein have been noted in animals on diets deficient in phenylalanine [1a] or lysine,[3] but alterations of this type were not found in rats fed leucine-deficient rations.[1b] The observations previously reported by us were made in acute experiments during which the animals were entirely deprived of a single essential amino acid so that their food consumption was markedly diminished and the animals rapidly lost weight. The anatomic alterations might be considered to be due in part at least to general malnutrition.

Histidine, the third amino acid to be studied, represents a heterocyclic type of amino acid. The two substances previously investigated,

From the Departments of Pathology and Physiological Chemistry, Wayne University College of Medicine.

* Frederick Stearns & Company Fellow in Physiological Chemistry.

1. Maun, M. E.; Cahill, W. M., and Davis, R. M.: (*a*) Arch. Path. **39**:294, 1945; (*b*) **40**:173, 1945.

2. Buschke, W.: Arch. Ophth. **30**:735, 1943.

3. Allen, E.: Sex and Internal Secretions, ed. 2, Baltimore, Williams & Wilkins Company, 1939, chap. 22.

phenylalanine[1a] and leucine,[1b] represent the aromatic and aliphatic types of amino acids, respectively. Although histidine is required for normal growth, it is synthesized in the adult rat and in man as evidenced by the fact that nitrogen balance can be maintained when this amino acid is missing from the diet.[4] Since histidine may be dispensable in the nutrition of adult rats, one might expect less manifest alterations in the health and the tissues of young animals restricted to diets completely devoid of this amino acid.

EXPERIMENTAL PROCEDURE

Animals.—Weanling rats of the Fisher line, 344 strain, which were 30 days old, were pair-fed throughout the experiment in individual cages. All of the eleven pairs of rats survived the twenty-eight day experimental period and were then killed by decapitation without anesthesia.

Diets.—The rats were fed purified diets [1a] of crystalline amino acids, crystalline vitamins, fats and sucrose plus the necessary salts. The adequate control diets contained 6.4 Gm. of histidine per kilogram, which was replaced in the deficient diets by 6.4 Gm. of sucrose. The average food consumption per rat in both groups was 2.98 Gm. per day.

Autopsies.—Examinations were made immediately after the animals were killed, each organ being routinely inspected and weighed. The entire organ or a portion thereof was fixed in Zenker's fluid, and one each of the paired organs with the exception of the eyes was fixed in Bouin's solution. Additional sections were fixed in a 4 per cent solution of formaldehyde. The tissues other than the eyes were embedded in paraffin, sectioned at 6 microns and stained with hematoxylin and eosin. Other stains were employed in particular instances as necessity demanded.

RESULTS

Health.—Animals consuming the deficient diets showed more lassitude than the control rats; the former appeared to sleep most of the time, and it was necessary to stimulate them to activity. The deficient animals showed no signs of hunger when fed, and near the close of the experimental period their coats appeared shaggy. The observed alterations were, however, much less pronounced than those that had been previously noted in the experiments in which the rats were fed phenylalanine-deficient or leucine-deficient diets.

Changes in Weight.—The animals fed deficient diets showed an average loss of weight of 10.2 Gm. each, whereas the control animals lost an average of 0.7 Gm. each. It is noteworthy that every rat on a histidine-free diet lost weight, while some of the control animals failed to lose weight although the caloric value of their diets equaled that of the experimental diets (figure).

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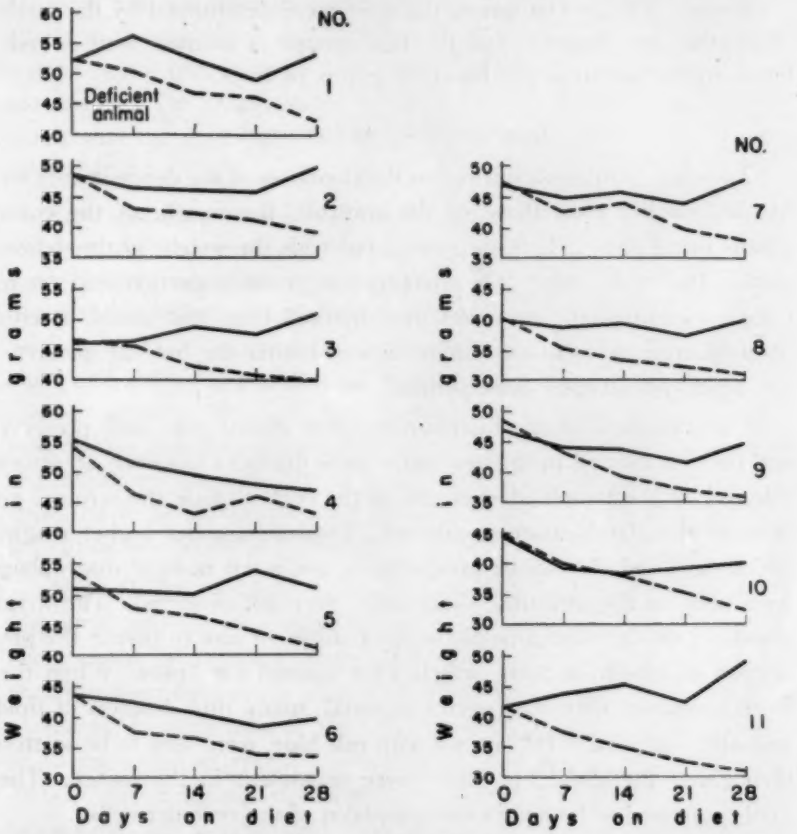
4. Rose, W. C.; Haines, W. J.; Johnson, J. E., and Warner, D. T.: J. Biol. Chem. **148**:457, 1943. Burroughs, E. W.; Burroughs, H. S., and Mitchell, H. H.: J. Nutrition **19**:363, 1940.

Organ Weights.—Each organ was weighed immediately after its removal, and the percentage of the organ weight was determined. (The percentage of an organ weight $= \frac{\text{organ weight}}{\text{body weight}}$). The weights were compared in each experiment, and the totals were submitted to statistical analysis. The only consistent variation was noted in the thymuses.

Roentgen Studies.—Several days prior to the end of the experimental period, roentgen studies were made of the complete skeletons of both the



The more rapid loss of weight of histidine-deficient rats as compared with their pair-fed controls. The broken line represents the deficient rat in each pair.

control and the deficient animals. There were no differences in the two groups.

Hemoglobin.—Determinations of the hemoglobin content of the blood were made by the Sheard-Sanford method [5]; the hemoglobin was found

5. Sheard, C., and Sanford, A. H.: J. Lab. & Clin. Med. **14**:558, 1929.

to be consistently lower in the deficient animals, which averaged 10.4 Gm. per hundred cubic centimeters, whereas the control animals averaged 14.1 Gm. pr hundred cubic centimeters.

Plasma Proteins.—Total plasma proteins were determined by the micro-Kjeldahl method with the aid of a Pregl[6] distillation apparatus. Only slight alterations were found, the average for the histidine-deficient rats being 5.42 Gm. per hundred cubic centimeters, compared with 6.19 Gm. for the control animals.

Hepatic Fats.—The fats of the liver were determined by the method of Leathes and Raper,[7] and the two groups of animals were found to have similar amounts per hundred grams of liver.

MORPHOLOGIC OBSERVATIONS

Thymus.—On gross inspection the thymuses of the deficient rats were notably smaller than those of the controls, the weight of the control glands being 48.9 ± 11.9 mg., compared with the weight of the deficient glands 14.3 ± 2.3 mg. The atrophy, on gross inspection and on histologic examination, was much less marked than that noted in either phenylalanine-deficient or leucine-deficient animals, but the pattern of the observed atrophy was similar.

The structure of the histidine-deficient glands was well preserved, and the cortical and medullary zones were distinct; however, an obvious thinning of the lymphoid elements of the cortex made the stromal portions of the glands more prominent. Inspection under higher magnification disclosed fibroblastic proliferation, and a few nests of macrophages were seen in the medulla. Giant cells were not observed. The thymic glands of the deficient animals were of sufficient size to permit the preparation of frozen sections, which were stained for lipids. When these were compared with the control material, many tiny droplets of lipids, stainable with sudan III but not with nile blue, were seen to be scattered throughout the gland but were more prominent in the cortex. These lipids appeared to be within the cytoplasm of the reticular cells.

Adrenal Glands.—The glands from both groups of animals were comparable in weight. Examination of the prepared sections stained with hematoxylin and eosin showed little evidence of compression of the middle zone or cellular atrophy such as that previously noted in the

6. Pregl, F.: Die quantitative organische Mikroanalyze, ed. 2, Berlin, Julius Springer, 1912.

7. Leathes, J. B., and Raper, H. S.: The Fats, ed. 2, New York, Longmans, Green & Co., 1925.

phenylalanine-deficient and leucine-deficient rats. Frozen sections of these glands were stained with sudan III, and on examination the adrenal glands of the histidine-deficient rats showed a slight decrease of the lipids in the zona glomerulosa and in a few patchy areas in the outer portion of the middle zone, but the lipids stainable with sudan III proved to be almost equal to those seen in the adrenal glands of the control animals.

Hypophysis.—The hypophyses from the control and the histidine-deficient rats showed no significant differences in weight. After the glands had been fixed in Zenker's solution, sections were made and stained with hematoxylin and eosin, Mallory's trichrome stain and azocarmine. On examination of the sections, no cellular changes were found, and the distribution of cells was similar in the deficient and the control animals.

Bone and Marrow.—Examination of sections of bone taken from the sternum and the vertebrae and of longitudinal sections of long bones showed alterations similar to, but less pronounced than, those previously described in animals maintained on phenylalanine-deficient and leucine-deficient diets. The epiphyses were narrowed, and the trabeculae were blunt and short. The degree of calcification was equal to that found in control animals, although the calcified areas were observed to stain irregularly. Detailed studies of the marrow were not possible because of unsatisfactory preservation of the marrow elements; however, there appeared to be no pronounced alterations between those of the control and those of the deficient group.

Male Genital System.—On gross inspection the testes of the two groups of animals were comparable in appearance and were found to be about equal in weight. Examination of the prepared sections revealed that the spermatogenesis of the histidine-deficient animals was delayed as compared with that of the control animals but was more advanced than that noted in rats fed phenylalanine-deficient and leucine-deficient diets. Spermatogonia and primary spermatocytes could be readily distinguished, but degenerated cells often occluded the lumens. Mitoses were abundant in the control animals, and in a few instances spermatids were seen, but mature spermatozoa were not noted in any of the deficient animals. The interstitial cells were unaltered in both groups. Examination of the prostates and seminal vesicles showed them to be comparable in the histidine-deficient and the control animals.

Eyes.—During the experiment, the eyes of the animals were repeatedly inspected, but no distinct gross differences were found. After the

eyes had been fixed in Zenker's fluid, they were embedded in celloidin and cut at 5 microns. The sections were stained with hematoxylin and eosin and with Mallory's trichrome stain. The eyes from the control animals were essentially normal.

The changes previously noted in leucine-deficient rats were also present but were less striking in rats on histidine-deficient diets. These consisted of thinning and stratification of the corneal epithelium with keratin formation on the surface. The substantia propria appeared thickened, but the structural pattern was well preserved. The vessels immediately beneath the corneal epithelium were dilated and prominent, and in a few instances scattered leukocytes were present in the perivascular tissue. Descemet's membrane was more prominent than in the control animals, but the anterior chambers were free of exudates. The structure of the remainder of the eyes was normal.

Other Organs and Tissues.—The remaining organs were inspected and, when it was feasible, weighed and sections of each were prepared for examination. All sections were stained routinely with hematoxylin and eosin. Best's carmine stains were used for the liver specimens and cresyl violet was employed to stain tissues of the central nervous system. The following tissues and organs of the histidine-deficient rats, when compared with the control animals, were found to present no significant alterations: heart, lungs, liver, spleen, pancreas, kidneys, voluntary muscle, skin, urinary bladder, salivary glands, thyroid gland, parathyroid glands, female reproductive system and the central and peripheral nervous system.

COMMENT

Animals fed histidine-deficient diets lost more weight than did their pair-fed partners, but the deficient rats appeared healthier than rats maintained on either phenylalanine-deficient or leucine-deficient diets. It is noteworthy that rats on the diets free of histidine had low hemoglobin (averaging 10.4 Gm. per hundred cubic centimeters of blood, which is comparable with the hemoglobin value found in phenylalanine deficiency). As might be predicated, since the growing rat is able to synthesize some of its required histidine, the anatomic alterations were similar to, but less evident than, those observed in other amino acid deficiencies. The experiment recorded here may have been too brief to permit the development of extensive anatomic alterations.

SUMMARY

Young inbred rats were fed purified diets made of crystalline amino acids, crystalline vitamins, dextrin, hydrogenated cottonseed oil (crisco) and the necessary salts. The animals were pair-fed so that the food con-

sumption of the control animals was equal to that of the deficient animals whose diet was devoid of histidine. The deficient animals lost weight more rapidly throughout the experiment and were less active than the control animals. At the end of a twenty-eight day experimental period the histidine-deficient group showed decrease in hemoglobin, atrophy of the thymus and vascularization and epithelial metaplasia of the cornea.

OCCURRENCE OF RHEUMATIC CARDITIS IN THE NATIVE POPULATION OF CURAÇAO, NETHERLANDS WEST INDIES

PHILIP H. HARTZ, M.D.
Pathologist of the Public Health Service
AND
ARY VAN DER SAR, M.D.
Internist of the Public Health Service
CURAÇAO, NETHERLANDS WEST INDIES

It seems to be a widely accepted belief, at least in the American medical literature, that acute rheumatic fever and rheumatic carditis are extremely rare or do not occur in the tropics. According to MacCallum,[1] the disease is hardly to be found in the tropics. Boyd,[2] discussing the etiologic factors of acute rheumatic fever, cited Coburn, who pointed out that the incidence of acute rheumatic fever parallels the incidence of streptococcic diseases, such as scarlet fever, and that both are almost unknown in the tropics. A child suffering from recurrent attacks of rheumatic fever and streptococcic sore throat in the slums of New York should remain well when transported to South America. Forbus,[3] also citing Coburn, observed that "it has been known for a long time that acute rheumatic fever is a disease of temperate climates and is almost unknown in tropical regions except in areas of high altitude where the climate is severe." Steinbröcker [4] advised removal of patients with rheumatic carditis to the southern states and Puerto Rico. According to Hegler,[5] acute rheumatic fever is rare in the tropics. It is almost completely absent in the Antilles and Puerto Rico but more frequent in other tropical countries, e. g., India. Strong [6] stated that from a study of the statistical reports and from the writings of various authorities there would seem to be two cosmopolitan diseases which are of extreme rarity in natives in the tropics: rheumatic fever and scarlet fever. He further pointed out that although 614 cases of rheumatic fever with one death were reported from the Gold Coast in 1911, there was no increase in admissions for vascular disease as would naturally be expected. Strong cited Manson-Bahr, who stated that there are some who never observed rheumatic fever or endocarditis in a life-long experience of India, Malaya, South China and Central Africa. He also cited MacKinnon, according to whom chorea was never observed in East African children, though Chesterman found this disease occasionally in Central Africa.

There are a few reports in which the occurrence of acute rheumatic fever in the tropics is mentioned. Denecke [7] mentioned the occurrence of the sequelae of rheumatic carditis in Fernando Po (off West Africa) and Rio Muni, though it is not stated how this diagnosis was made. He did not observe scarlet fever. In the Netherlands East Indies clinical acute rheumatic fever has been observed a few times; 1 case of mitral endocarditis is mentioned.[8] We did not find autopsy reports.

Statements to the effect that certain diseases do not or only rarely occur in tropical countries must always be viewed with a certain suspicion. Statistics based on clinical observations alone are unreliable. Only when autopsies with histologic examinations are regularly performed in sufficient numbers and when the eventual differences between the general and the hospital population are taken into consideration, can the incidence of certain diseases in the population of a given region be determined approximately.

1. MacCallum, W. G.: A Textbook of Pathology, ed. 7, Philadelphia, W. B. Saunders Company, 1941, p. 788.

2. Boyd, W.: (*a*) The Pathology of Internal Diseases, ed. 4, Philadelphia, Lea & Febiger, 1944, p. 10; (*b*) A Text Book of Pathology, ed. 4, ibid., 1943, p. 154.

3. Forbus, W. D.: Reaction to Injury, Baltimore, Williams & Wilkins Company, 1943, p. 231.

4. Steinbröcker, O.: Arthritis in Modern Practice, Philadelphia, W. B. Saunders Company, 1942, p. 120.

5. Hegler, C.: Der acute Gelenkrheumatismus, in von Bergmann, G., and Staehelin, R.: Handbuch der inneren Medizine, ed. 3, Berlin, Julius Springer, 1934, vol. 1, p. 159.

6. Strong, R.: Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases, ed. 6, Philadelphia, The Blakiston Company, 1942, vol. 2, p. 1605.

7. Denecke, K.: Arch. f. Hyg. **126**:331, 1941; abstracted, Trop. Dis. Bull. **39**:348, 1942.

8. Hidayat, D.: Geneesk. tijdschr. v. Nederl.-Indië **72**:1548, 1932. Groot: ibid. **76**:1588, 1936.

This applies not only to cancer [9] but also to other diseases, especially when the etiologic factor, as in acute rheumatic fever, is unknown and when the diagnosis can be confirmed only by histologic examination.

As it is the purpose of this paper to demonstrate that rheumatic carditis is not so rare among the natives of Curaçao, a tropical island, some facts about the geographic position and climate of this island are not out of place. Curaçao lies in the Caribbean Sea between 12° 2′ 18″ and 12° 23′ 30″ northern latitude. The shortest distance between the island and the coast of South America (Venezuela) amounts to 38 sea miles. The yearly main temperature is 81.5 F. The average annual rainfall is 21.7 inches (55 cm.), though annual rainfalls of 40 inches (101.5 cm.) and more have been recorded. The rainy season includes the months of October, November, December and January. The relative humidity is ± 73 per cent. In the neighboring islands Aruba and Bonaire the same climatic conditions prevail.[10]

Clinically, acute rheumatic fever is not infrequently observed among the native population of Curaçao.[11] Among 3,391 admissions for internal diseases during the years from 1940 to 1945 there were 61 for acute rheumatic infection. In 20 cases there was acute arthritis, in 30 cases arthritis combined with endocarditis and in 11 cases endocarditis without arthritis (see table). In 3 patients (2 women, 50 and 56 years old and 1 man 40 years old) the disease changed into secondary chronic rheumatism. In the same period 3 cases of typical chorea minor were observed: 2 cases concerned 14 year old girls; one recovered without complications; the other died, and at autopsy endocarditis verrucosa of the mitral valve was found (see report of case 20). The third case concerned a 5 year old girl; four weeks before admission she had suffered an attack of acute rheumatic fever; she died of cardiac decompensation.

Streptococci are regularly isolated from throat swabs; of 124 cases in which throat swabs were examined in the bacteriologic laboratory of the Public Health Service (Dr. A. W. Pot), hemolytic streptococci were found in 40, anhemolytic streptococci in 9 and Streptococcus viridans in 9. We did not observe scarlet fever.

9. Bonne, C.: (*a*) Am. J. Cancer **25**:811, 1935; (*b*) **30**:435, 1937. (*c*) Hartz, P. H.: ibid. **40**:355, 1940.

10. Realino, F. M.: De Nederlandsche Antillen, ed. 3, Curaçao, St. Thomas College, 1938.

11. van der Sar, A.: Rev. Policlin. Caraças **12**:213, 1943.

Since February 1936 1,481 persons have been examined post mortem on Curaçao. This number includes 174 Chinese, Syrians, Dutch and other nationals; the remainder were in the main natives of Curaçao and a small number of Aruba and Bonaire, where, as already stated, the same climatic conditions prevail. Among these 1,307 autopsies there were 20 in which a gross diagnosis of rheumatic carditis or of the sequelae of rheumatic carditis was made. In 12 of these a histologic examination could be made, and in 11 out of these 12 instances typical Aschoff bodies were found.

REPORT OF CASES

1. Negro girl, 12 years old. Clinical data were not available. The autopsy diagnosis was: verrucous endocarditis of the mitral valve; cardiac hypertrophy and dilatation; passive congestion of the lungs and of the liver. The heart weighed 325 Gm. Microscopic examination of the heart showed typical Aschoff bodies.

2. Negro girl, 9 years old. Clinical data were not available. The autopsy diagnosis was: verrucous endocarditis of the mitral valve; myocarditis; fibrinous pericarditis. Microscopic examination of the heart showed typical Aschoff bodies in the myocardium and the pericardium.

3. Negro woman, 24 years old, who died during transport to the hospital. The autopsy diagnosis was: mitral stenosis; chronic inflammation of the mitral and aortic valves; dilatation of the left atrium; thrombosis of the right auricle. The heart weighed 250 Gm. Microscopic examination of the heart showed typical Aschoff bodies.

4. Negro girl, 9 years old, with a history of acute rheumatic fever (polyarthritis). The autopsy diagnosis was: verrucous endocarditis of the mitral valve; fibrinous pericarditis; cardiac hypertrophy and dilatation; thrombosis of the right auricle; brown induration of the lungs. The heart weighed 245 Gm. Microscopic examination of the heart showed Aschoff bodies.

5. Negro boy, 15 years old. Clinical data were not available. The autopsy diagnosis was: endocarditis of the mitral and aortic valves; myocarditis; thrombosis of the right auricle; cardiac hypertrophy and dilatation; adhesive pericarditis; passive congestion of the lungs and of the liver. The heart weighed 365 Gm. Microscopic examination of the heart showed typical Aschoff bodies, especially in the right auricle.

6. Negro boy, 8 years old with a history of acute rheumatic fever (polyarthritis). The autopsy diagnosis was: chronic inflammation of the mitral valve; cardiac hypertrophy and dilatation. The heart weighed 210 Gm. Microscopic examination of the heart showed typical Aschoff bodies in the myocardium and the pericardium.

7. Negro girl, 6 years old, with no history of acute rheumatic fever (polyarthritis) or tonsillitis. On admission an electrocardiogram revealed a P-R interval of 0.16 second; the P-R waves were not enlarged or doubled. During her stay in the hospital a 2:1 block developed, which after a few days was replaced by an intraventricular block; the duration of QRS was 0.12 second. The autopsy diagnosis was: endocarditis verrucosa of the mitral valve; myocarditis; fibrinous pericarditis; cardiac hypertrophy and dilatation; passive

congestion of the internal organs. Microscopic examination of the heart showed large Aschoff bodies in the myocardium and the pericardium.[11a]

8. Negro girl, 9 years old, with acute rheumatic fever (polyarthritis) on admission and in the history. Cultures were made from the blood and from throat swabs, but no streptococci were found. The autopsy diagnosis was: serofibrinous pericarditis, the pericardial sac containing 300 cc. of exudate; chronic endocarditis of the mitral valve; passive congestion of the lungs. The heart weighed 255 Gm. Microscopic examination of the heart showed typical Aschoff bodies, especially in the right auricle.

9. Arubian girl, 17 years old, with no history of acute rheumatic fever. She was admitted because of pulmonary tuberculosis. The autopsy diagnosis was: pulmonary tuberculosis; tuberculous peritonitis; caseous salpingitis; verrucous endocarditis of the mitral and aortic valves; small myocardial scars. Microscopic examination of the heart showed a few typical Aschoff bodies.

10. Negro girl, 10 years old. On admission she had had typical acute rheumatic fever for two days. There was no history of rheumatic fever in the family. There had been typical symptoms of mitral insufficiency. An electrocardiogram showed a P-R interval of 0.22 second, P waves of normal interval and amplitude, and a QRS complex of normal duration and form. The blood culture was negative; a culture of material from the throat gave growths of an anhemolytic streptococcus and a pneumococcus; a culture from a throat swab at a later date gave exclusively a growth of an anhemolytic streptococcus. The autopsy diagnosis was: endocarditis verrucosa of the mitral, tricuspid and aortic valves; myocarditis; cardiac hypertrophy, especially of the left ventricle. The heart weighed 275 Gm. Microscopic examination of the heart showed typical Aschoff bodies in great numbers and numerous small necroses in the papillary muscles of the left ventricle.

11. Negro boy, 12 years old. He was first admitted in June 1943, one day after the appearance of the first symptoms and two days after his sister died of rheumatic fever, chorea minor and mitral insufficiency. He was discharged in fairly good condition after two and a half months. He was readmitted in May 1945 with shortness of breath and retrosternal pain. For two days he had suffered from fever and painful joints. There was marked dilatation of the heart; the teleroentgenogram gave a diameter of 195 mm. There were systolic and diastolic murmurs. The blood culture gave a negative result, and no streptococci could be isolated from a throat swab. Death occurred seven days after admission. The father of the patient had suffered from rheumatic fever at the age of 11 years. From the throats of the father (now 49 years old) and of 3 of 4 brothers and sisters of the patient (respectively, 3, 5 and 18 years old) anhemolytic streptococci were isolated; not, however, from the throat of the sister who died of acute rheumatic fever. The autopsy diagnosis was: fibrinous pericarditis; recurrent verrucous endocarditis of the mitral and aortic valves; mitral insufficiency; marked cardiac hypertrophy and dilatation, especially on the left side. The heart weighed 430 Gm. Microscopic examination of the heart showed typical Aschoff bodies.

12. Arubian woman, 44 years old, who died after a supravaginal hysterectomy, with the symptoms of pulmonary embolism and infarction. The autopsy diagnosis was: chronic endocarditis of the mitral and aortic valves; mitral stenosis; dilatation of the left atrium and of the right ventricle; small myocardial scars; pulmonary embolism and infarction. The heart weighed 470 Gm. No Aschoff bodies were found at microscopic examination.

Cases of Acute Rheumatic Infection in Curaçao 1940 to 1945

Diagnosis	Distribution of Cases by Age and Sex												Male	Female	Total	Deaths
	1-5 Yr.	6-10 Yr.	11-15 Yr.	16-20 Yr.	21-25 Yr.	26-30 Yr.	31-35 Yr.	36-40 Yr.	41-45 Yr.	46-50 Yr.	51-55 Yr.	56-60 Yr.				
Acute rheumatic fever (arthritis)	..	1	2	2	2	4	2	1	..	1	4	1	13	7	20	0
Acute rheumatic fever (arthritis) and endocarditis	2	6	8	1	1	5	1	2	1	1	1	1	13	17	30	9
Endocarditis.....	1	..	..	6	..	1	2	..	1	..	..	..	3	8	11	5
Total.....	3	7	10	9	3	10	5	3	2	2	5	2	29	32	61	
Deaths.....	2	4	2	2	..	2	..	..	1	..	..	1	7	7		14

In the following cases no tissue was available for microscopic examination.

13. Negro woman, 23 years old. The autopsy diagnosis was: chronic endocarditis of the mitral valve; myocarditis; hypertrophy of the left ventricle.

14. Negro man, 20 years old. The autopsy diagnosis was: chronic endocarditis of the mitral and aortic valves; serofibrinous pericarditis; hypertrophy and dilatation of the left ventricle; brown induration of the lungs.

15. Negro woman, 26 years old, whose death occurred one day after delivery of her infant. The autopsy diagnosis was: chronic endocarditis of the mitral and aortic valves; mitral stenosis, hypertrophy and dilatation of the right ventricle and of the left atrium.

16. Negro woman, 30 years old. The autopsy diagnosis was: chronic endocarditis of the mitral valve; mitral stenosis; verrucous endocarditis of the tricuspid valve; dilatation of the left atrium and of the right ventricle; hypertrophy of the right ventricle.

17. Negro woman, 42 years old. The autopsy diagnosis was: recurrent endocarditis of the mitral valve;

11a. Since this paper was submitted a 7 year old brother of this patient has died. Examination revealed rheumatic carditis with numerous Aschoff bodies.

mitral stenosis; dilatation of the left atrium, the right atrium and the right ventricle; brown induration of the lungs.

18. Negro woman, 26 years old, whose death occurred twenty minutes after delivery. The autopsy diagnosis was: chronic endocarditis of the mitral valve; dilatation of the left atrium; hypertrophy of the right ventricle.

19. Negro girl, 19 years old. The autopsy diagnosis was: fibrinous pericarditis; chronic endocarditis of the mitral and tricuspid valves; myocarditis.

20. Negro girl, 14 years old, with chorea minor. The autopsy diagnosis was: hemopericardium; fibrinous pericarditis; chronic endocarditis of the mitral valve; cardiac hypertrophy. The heart weighed 360 Gm.

COMMENT

Although the etiologic nature of acute rheumatic fever is still an unsolved problem despite the several theories which are being discussed, there exists a characteristic lesion, the Aschoff body, by which rheumatic carditis can be recognized with great certainty.[12] Its occurrence in patients in whom a rheumatic infection could be excluded has never been conclusively demonstrated.[12] We believe, therefore, that by the finding of the typical Aschoff bodies the diagnosis of rheumatic carditis has been sufficiently established in cases 1 to 11. In case 12 no Aschoff bodies were found, and in cases 13 to 20 no tissue was available for microscopic examination. Nevertheless a rheumatic origin for the cardiac lesions observed in these cases at autopsy is very probable. These lesions were all typical of rheumatic carditis. The fact that no Aschoff bodies were found in case 12 does not exclude rheumatic fever; these bodies are finally transformed into scar tissue, which in this case is the more probable since the patient was already in the fifth decade of life; moreover, Aschoff bodies may be absent in rheumatic fever.[2] Applicable to cases 12, 16 and 17 is the opinion of Carey Coombs [13] that all cases of mitral stenosis are rheumatic in origin. Furthermore, in view of the high percentage (90 per cent) in which Aschoff bodies were found in the first 12 cases, it is reasonable to suppose that microscopic examination would have yielded several more cases with these bodies, especially among those in which acute pericarditis and verrucous endocarditis were present at autopsy.

It follows from the foregoing report of cases that rheumatic carditis does occur on Curaçao, a tropical island in the Caribbean Sea, and that its occurrence is certainly not rare. Apart from the negative statements from Puerto Rico we could not obtain data as to the occurrence of this disease on other Caribbean islands. It is, however, interesting to note that Jaffé [14] only twice observed rheumatic carditis in his large autopsy material in Caracas (Venezuela).

Several other facts have to be noted: Nearly all of our patients belonged to the poorer classes, whose intake of vitamin C is certainly not high, though they did not show any symptoms of avitaminosis and we never observed scorbutic conditions in our clinical material; on Curaçao the incidence of acute rheumatic fever does not parallel that of scarlet fever, which is really exceedingly rare on Curaçao, when it occurs at all; different kinds of streptococci are being isolated from throat swabs, but only rarely from the throats of patients suffering from acute rheumatic fever; cultures of blood from the patients were always negative. In 1 case [9a] there was a marked familial incidence of the disease.

The conclusion to be drawn from our observations is that statements concerning the rareness of rheumatic carditis in the tropics are probably too general; it is to be expected that when reliable data, especially reports of autopsies with microscopic examinations, become available, acute rheumatic fever and rheumatic carditis will be found to occur more frequently in other tropical regions. It is of course possible that tropical countries exist where acute rheumatic fever and rheumatic carditis are really rare or where they do not occur [15]; we believe, however, that in this case the rareness or nonoccurrence of these diseases is not due to the tropical conditions per se but to local circumstances or perhaps racial peculiarities, which also play an important role in the incidence and course of other diseases. It must be considered possible that the incidence of acute rheumatic fever is far less influenced by climatic factors than is commonly stated. Theories based on the alleged parallelism between the incidence of scarlet fever and that of acute rheumatic fever are not in accordance with the facts. Finally, physicians sending patients with rheumatic carditis to a tropical country should try to obtain reliable information about the incidence of rheumatic carditis in that country and not rely on climatic data alone.

SUMMARY

Among 3,391 admissions for internal diseases at Curaçao, Netherlands West Indies, over a

12. Saphir, O.: Arch. Path. **32**:1000, 1941.
13. Carey Coombs, cited by Boyd.[2a]
14. Jaffé, R.: Personal communication to the authors.
15. Rogers, L., and Megaw, J. W. D.: Tropical Medicine, ed. 5, London, J. & A. Churchill, Ltd., 1944, pp. 480 and 481.

period of five years, there were 61 for acute rheumatic fever. Three cases were complicated by chorea minor. Scarlet fever was not observed. Among 1,307 autopsies on natives of Curaçao, Aruba and Bonaire there were 20 which disclosed typical gross lesions or sequelae of rheumatic carditis. In 12 of these histologic examination was possible, and in 11 typical Aschoff bodies were found.

The supposition is advanced that rheumatic carditis is more frequent in the tropics than is commonly believed but that the true incidence can be determined only by collecting reliable data, based especially on autopsies with histologic examinations.

FAT NECROSIS STUDIES

VI. The Effect of Feeding Lipase-Containing Vegetable Seed on the Production of Fat Necrosis

M. PINSON NEAL, M.D.
COLUMBIA, MO.

SOME features of fat necrosis remain baffling. This is largely due to the fact that the lesion occurs under variable conditions and in different locations. It is best considered under the terms: (1) "spontaneous," (2) "experimental," (3) "pancreatic" and (4) "traumatic" or "subcutaneous." The first two terms are self explanatory, and either one is frequently combined with one of the other terms for better designation.

Spontaneous pancreatic fat necrosis may be caused by any process or occurrence that enables pancreatic juice to escape from its natural channels. It does not occur by absorption of that lipase-containing substance from these normal passages. It has followed trauma, acute inflammation and cancer of the pancreas; inflammation, tumor and parasitization of the pancreatic ducts; lithiasic, inflammatory, parasitic or neoplastic obstruction of the ampulla of Vater. The lesions are found within the abdominal fatty tissues unless the enzyme gains access directly to other fat deposits, such as those in the subcutaneous tissue, by way of wounds or drainage tracts.

Experimental fat necrosis has been produced by ligation of the pancreatic ducts, temporary obstruction of the pancreatic vascular circulation, sectioning of the pancreas, placing of fresh pancreatic tissue into the abdominal cavity, and intraperitoneal injections, in both cold-blooded and warm-blooded vertebrates, of fresh pancreatic juice, emulsions of pancreas of various animals, commercial pancreatin and lipase derived from both animal and vegetable sources.[1]

Traumatic or subcutaneous fat necrosis is a fatty tissue change which has the characteristics of pancreatic fat necrosis but results from mechanical trauma of subcutaneous adipose tissue. It is seen principally in the fat of the female mammary gland and in the subcutaneous fat of the newborn. In the latter it is incident to delivery and has been termed sclerema neonatorum. The trauma is responsible for cell death and the consequent liberation of cell-contained lipase, which acts locally

From the Department of Pathology, Bacteriology and Preventive Medicine, University of Missouri School of Medicine.

1. Neal, M. P., and Ellis, M. M.: South. M. J. **23**:313, 1930.

on fat. Farr [2] recorded that by pinching the skin and subcutaneous tissue of young pigs with forceps he was able to produce this type of fat necrosis and stated that no pancreatic disease or injury is necessary.

Some writers have been wont to explain the occasional finding of fat necrosis in pericardial, mediastinal, mammary and subcutaneous adipose tissue as of pancreatic origin. They have theorized that lipase of pancreatic source is transplanted by the blood to these sites, where, for an unexplained reason, it acts. One logically asks, Why the particular localization? Why not a more general involvement of fatty tissue, and why do other fat tissues escape?

While the traumatic or subcutaneous type of fat necrosis is of interest and creates a problem in itself, this article is concerned primarily with fat necrosis of the so-termed pancreatic origin, meaning that it is the result of the escape of lipase from some portion of the normal passageway for pancreatic secretion or the introduction of an active lipase-containing substance into living animals.

SPONTANEOUS FAT NECROSIS IN ANIMALS

Horvath and Chang [3] reported that in rabbits after prolonged feeding and overconsumption of lipase-containing raw soybeans, they discovered in the perirenal fat, on killing the animals, a lesion which was interpreted as fat necrosis. Soybeans heated until the lipase had been destroyed, according to the ethyl butyrate test, fed to like animals and in similar quantities gave no such lesions in fat tissue. This led to the conclusion that the substance activating the fat tissue change was destroyed by heat. Horvath [4] had previously noted similar necrotic areas in the perirenal fat and sometimes in the subpleural fat of beef cattle in northern China that had been fed heavily on soybean cake and black soybeans (observed in the slaughter house in Tsingtau, China). The lesions in both the rabbits and the beef cattle were interpreted as fat necrosis, and the fact that rabbits fed on cooked soybeans did not show the necrosis was suggestive of an enzyme as the cause. Horvath [4] did not observe the condition in Mongolian cattle fattened on grass (examined in Tientsin).

Horvath and Chang [3] gave no detailed description of the lesion or statement as to the criteria on which they arrived at the conclusion "fat necrosis." There is nothing in their report to indicate histologic, bacteriologic or parasitologic studies of the material.

It is difficult to accept the idea of lipase consumed as a food being removed from the gastrointestinal tract to remote fat deposits and there localized as the factor in these lesions that have been recorded

2. Farr, C. E.: Ann. Surg. **77**:513, 1923.
3. Horvath, A. A., and Chang, H. C.: Am. J. Physiol. **78**:224, 1926.
4. Horvath, A. A., cited by Horvath and Chang,[3] p. 232.

as fat necrosis. Lipase is present and available in the pancreatic secretions that enter the duodenum. Certainly if it were absorbable from feeding sources, as from lipase-containing soybeans or peanuts, in sufficient quantities to cause fat necrosis, it would be absorbable also from the pancreatic source.

Lipase has an affinity for fat tissue, but one cannot explain why the foci of fat necrosis should be localized, for instance, in the perirenal or some other area. The characteristic fat necrosis occasionally seen in subcutaneous areas and in the breast is to be attributed not to localization of lipase derived from the intestinal tract but to liberation of lipase from cells in the area where the necrosis occurs as a result of some local damage, as that caused by trauma.

Differential Diagnosis.—It is well known among pathologists that foci of fat necrosis are readily confused with, and must be differentiated from, foci of tuberculosis, syphilis and cancer, from reactions to certain parasites, from foci of infection, from lesions incident to the injection of oils or following trauma and particularly from foci of postmortem change due to enzyme action on fatty tissues near the pancreas. Caution is especially called for in the interpretation of small white spherical lesions in animals where parasites are common. Neal,[5] in examining the carcasses of hogs that had been fattened on raw peanuts, found parasites of the species Stephanurus dentatus, commonly known as the kidney worm of swine, in numbers of these animals. Of particular significance was their presence in the pancreas and the pancreatic duct where, by occluding the duct system, they served as a cause of stagnation. Small lesions, 2 to 4 mm. in diameter, produced by the presence of these parasites or of their ova were on gross inspection differentiated with difficulty from fat necrosis. They were especially found in the peripancreatic, perirenal and periureteral fat deposits. The distinguishing and differential feature was that the parasites or their ova were present within the necrotic-appearing foci. Effort has been made, but without success, to develop a chemical stain that when applied to the gross lesion would give a differential or a selective reaction such as that used in demonstrating amyloid degeneration.

PROBLEM

The present studies were undertaken because it had been claimed [6] that the feeding of rabbits and cattle on raw soybeans produced fat necrosis and because our previous efforts [7] at producing this condition

5. Neal, M. P.: South. M. J. **34**:153, 1941.

6. Horvath and Chang.[3] Horvath.[4]

7. Neal, M. P., and Ellis, M. M.: Am. J. Clin. Path. **1**:251, 1931; J. Missouri M. A. **32**:37, 1935; footnote 1.

in experimental animals had failed except when concentrated and purified lipase, pancreatic secretion, emulsified fresh pancreas or pancreatin was injected either subcutaneously or intraperitoneally.

PROCEDURE

First Experiment (feeding of rabbits on soybeans or peanuts [8]).—A group of 16 rabbits was fed exclusively for periods of from five to twenty days on lipase-containing, American-grown, Manchu type raw soybeans or Virginia grown raw peanuts, with 8 animals being fed one or the other product. Sixteen rabbits as controls were fed similar products that had been heated to a temperature of 120 C. for one hour to destroy the lipase, and 8 others were fed a regular diet, largely of green vegetables, alfalfa, oats and shorts. At designated five, ten, fifteen and twenty day periods, members of each group and their respective controls were killed and examined.

Second Experiment (a study of the carcasses of 8,324 peanut-fattened hogs [8]).—This study was conducted at two southern Georgia packing plants at the height of the seasonal peanut fattening period on animals that were self fed, pastured or grazed on mature peanuts in the field. It was impossible to ascertain the length of time that an individual hog might have been on this type of feed, but it varied from two or three weeks to four months, certainly adequate time for fat necrosis to develop. The animals varied in live weight from 60 to 600 pounds (27 to 272 Kg.) and in ages from 5 or 6 months to 2½ to 3 years (a few). The average was between 150 and 200 pounds (68 and 90.5 Kg.) and between 7 and 10 months of age. They were almost invariably slaughtered within from forty-eight to seventy-two hours after being taken from the farm and their place of feeding, and the entire carcass was personally and immediately inspected. If there was doubt concerning the nature of a gross lesion, histologic studies were consistently done.

Third Experiment (feeding of white rats on peanuts or soybeans).—Sixty grown white rats were employed in this study. Thirty were fed exclusively on unshelled, raw Virginia-grown peanuts and 30 on uncracked, raw, American-grown, Manchu type soybeans for periods of time varying from five to ninety-one days. Two animals of each group were killed and minutely examined for evidence of fat necrosis on each of the following days of the period of restricted feeding: fifth, eleventh, fifteenth, twentieth, twenty-fifth, twenty-ninth, thirty-fifth, fortieth, forty-fifth, fiftieth, fifty-fifth and sixtieth. One rat of each group was killed on each of the following days: sixty-fifth, seventieth, seventy-fifth, eighty-second, eighty-seventh and ninety-first.

Immediately after the rats were killed, the various subcutaneous, abdominal and intrathoracic fatty tissues were minutely examined for fat necrosis. Of the 60 rats, all lived until the predetermined date for killing, and all except 7 revealed abundant fatty tissues. The 7 were poor in fat deposits.

The times selected for killing rabbits and white rats were chosen because of knowledge previously acquired from studies on intraperitoneal and subcutaneous injections of lipase in various vertebrates. In these, grossly recognizable foci of fat necrosis were demonstrated as early as sixteen and a half hours and as late as eight days after single injections of the lipase extractives.[1]

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8. Neal, M. P.: Fat Necrosis Studies: V. The Effect of Soybean and Peanut Feeding on the Blood and Urine Lipase of Rabbits and in the Production of Fat Necrosis, South. M. J. **38**:793, 1945.

RESULTS

Of the 16 rabbits fed exclusively on raw soybeans or raw peanuts, those killed on the fifth, tenth, fifteenth and twentieth days showed no fat necrosis.

Among 8,324 peanut-fattened hogs, only 3 disclosed perirenal and 2 pancreatic fat necrosis. The presence of the kidney worm, Stephanurus dentatus, in the pancreas or the pancreatic duct, and within the kidney or the perirenal fat, was considered the cause of fat necrosis in these 5 hogs.

Thirty white rats fed exclusively raw peanuts and 30 others fed raw soybeans for periods of from five to ninety-one days failed to show fat necrosis when some of each group were put to death at essentially five day intervals.

SUMMARY

Under the conditions elsewhere described [8] fat necrosis is not produced in rabbits by the feeding of lipase-containing peanuts or soybeans.

Under the conditions of fattening, marketing and slaughtering of hogs, as previously reported,[5] fat necrosis is not produced by the feeding of lipase-containing peanuts. Fat necrosis was not produced in 60 white rats fed lipase-containing peanuts or soybeans.

Reports indicating that the ingestion of lipase-containing food, notably soybeans, is capable of producing fat necrosis in animals have not been confirmed by these studies. In fact, the evidence is that lipase in food may be discounted as a cause of fat necrosis.

Postmortem fatty tissue changes and those resulting from trauma, parasitization, infection and pancreatic lesions must be eliminated as factors before a food source of lipase is acceptable as producing this condition.

Spontaneous fat necrosis arising from conditions other than those that permit leakage of lipase from the normal pancreatic channels is also to be considered as the result of lipase but of lipase derived from some source other than that which enters the body by the way of the alimentary tract; i. e., lipase contained within food or forming a part of a food.

Interpretations based on gross inspection of the lesions alone are not dependable.

EXPERIMENTAL NEPHROPATHIES

IV. GLYCOSURIA IN DOGS POISONED WITH URANYL NITRATE, MERCURY BICHLORIDE AND POTASSIUM DICHROMATE

OPAL E. HEPLER, M.D., AND J. P. SIMONDS, M.D.
CHICAGO

Phosphatase is abundant in the superficial layers of the upper part of the intestine [1] and in the proximal convoluted tubules of the kidneys.[2] It is absent from the mucosa of the rectum and from the nephron from Henle's loop onward.[3] Glucose is absorbed rapidly from the duodenum and the jejunum [3] and in the proximal convoluted tubules of the kidneys.[4] It is absorbed very slowly from the rectum [5] and probably not at all in Henle's loop and the distal convoluted tubules. Numerous investigators [6] have therefore considered the possibility that renal phosphatase takes part in the absorption of glucose from the glomerular filtrate. Wilmer [7] has recently described a theoretic mechanism by which this enzyme may play a role in the absorption of sugar in the kidneys.

Although the literature dealing with the renal effects of the three inorganic chemical agents used in these experiments is extensive, relatively few investigators have included the possible production of glycosuria as one of the results. In 1913 Frank [8] injected subcutaneously into 5 rabbits 4 to 10 mg., and into 5 dogs 10 to 35 mg., of uranyl nitrate ($UrO_2[NO_3]_2$) and observed glycosuria in all of them. He injected intravenously into 7 rabbits 0.4 to 4.0 mg. of mercury bichloride ($HgCl_2$) and observed glycosuria in all of these animals. One dog given 50 mg. of potassium dichromate ($K_2Cr_2O_7$) by subcutaneous injection had 2 plus sugar in its urine. Milhorat and Deuel [9] produced glycosuria by injecting 15 mg. of uranyl nitrate per kilogram into each of 12 dogs, although the blood sugar levels in these animals never exceeded those in the normal controls. Both Frank [8] and Milhorat and Deuel [9] used much larger doses of the poisons than were employed in our experiments, which appear to stand alone as an attempt to determine the smallest dose capable of causing excretion of sugar in the urine. Major [10] studied fatal "chromic acid" poisoning in a human patient and observed that glycosuria appeared from time to time but that it bore no apparent relation to the amounts of blood sugar present. Mosenthal and Schlayer [11] reported glycosuria after poisoning with potassium dichromate.

RESULTS OF EXPERIMENTAL STUDY

The methods employed in these experiments have been described in a previous paper in this series.[12] Benedict's solution was used in testing the urine for sugar.

The urines of 54 dogs in our series [12] were examined for the presence of sugar after poisoning with various chemical agents, distributed as follows:

Uranyl nitrate	15 dogs
Mercury bichloride	17 dogs
Potassium dichromate	17 dogs
Phlorhizin	5 dogs
Total	54 dogs

The relation of the presence or the absence of glycosuria to other chemical and microscopic observations is presented in table 1.

There is a marked difference in the incidence of glycosuria in dogs poisoned with the three inorganic chemical agents used in these experiments. Two thirds of the dogs poisoned with uranyl nitrate had glycosuria, while this condition was found in less than one fourth (23.5 per cent) of those poisoned with mercury bichlo-

From the Department of Pathology, Northwestern University Medical School.

1. Grosser, P., and Husler, J.: Biochem. Ztschr. **39**:1, 1912.

2. Gomori, G.: Proc. Soc. Exper. Biol. & Med. **42**:23, 1939.

3. MacKay, E. M., and Clark, W. G.: Am. J. Physiol. **135**:187, 1941.

4. Smith, H. W.: The Physiology of the Kidneys, New York, Oxford University Press, 1937.

5. Houssay, B. A.; Foglia, V. G., and Fustinoni, O.: Endocrinology **28**:915, 1941.

6. (*a*) Dmochowski, A., and Assenhajm, D.: Naturwissenschaften **23**:501, 1935. (*b*) Kutscher, W., and Wolbergs, H.: ibid. **23**:559, 1935.

7. Wilmer, H. A.: Arch. Path. **37**:227, 1944.

8. Frank, E.: Arch. f. exper. Path. u. Pharmakol. **72**:387, 1913.

9. Milhorat, A. T., and Deuel, H. J.: Arch. Int. Med. **60**:77, 1937.

10. Major, R.: Bull. Johns Hopkins Hosp. **33**:50, 1922.

11. Mosenthal, H. O., and Schlayer: Deutsches Arch. f. klin. Med. **111**:127, 1913.

12. Simonds, J. P., and Hepler, O. E.: Arch. Path. **39**:103, 1945.

ride and potassium dichromate. The difference is even more pronounced when one compares the minimum dose of each agent required to produce glycosuria. Of 10 dogs that received single doses of 1 mg. or more of uranyl nitrate per hundred cubic centimeters of blood, 100 per cent had glycosuria. Two doses of 1 mg. each of potassium dichromate per hundred cubic centimeters of blood constituted the minimum amount of dichromate required to induce glycosuria. But even this was not constant, for only 4, or 44.4 per cent, of 9 dogs that received this dose had sugar in the urine. Three milligrams per hundred cubic centimeters of blood constituted the smallest dose of mercury bichloride to cause glycosuria; of 5 dogs that received this dose, only 4, or 80 per cent, had sugar in the urine.

All the dogs in this series that showed glycosuria also had albuminuria, except those receiving phlorhizin. But the converse is not true; that is, many of these dogs had albumin in the urine without glycosuria.

Blood for determination of sugar was taken in most instances from a vein in the leg without anesthesia. For technical reasons it became necessary with some animals to take blood from the inferior vena cava with the animal under ether anesthesia at the time the animal was killed. As was expected, the sugar content of each of these samples was very high. In table 2 a comparison is made between the blood sugar values before and after poisoning in those dogs from which blood was taken by a leg vein only. Within the limitation of this small group of 20 dogs, uranyl nitrate caused an increase in blood sugar with and without glycosuria, while potassium dichromate caused a rise in blood sugar chiefly in those dogs that had glycosuria. The highest value of sugar in blood from a leg vein was 125 mg. per hundred cubic centimeters, which is well below the threshold of dextrose. After poisoning with phlorhizin, on the other hand, the blood sugar was lower than the control values in the 3 dogs on which we have results. Lundsgaard,[13] Kastler,[14]

TABLE 1.—*Glycosuria in Dogs Poisoned with Uranyl Nitrate ($Uro_2[NO_3]_2$), Mercury Bichloride ($HgCl_2$), Potassium Dichromate ($K_2Cr_2O_7$) and Phlorhizin*

Dog	Dose, Mg. per 100 Cc. of Blood	Glycosuria	Albuminuria	Blood Phosphatase, Bodansky Units		Blood Sugar, Mg. per 100 Cc.		Kidney Phosphatase		Necrosis of Tubular Epithelium
				Before	After	Before	After	Chemical Bodansky Units	Microscopic	
K Ur 15	1 × 0.05 mg.	0	Trace	3.00	5.00	97.0	174.0*	16.06	++++	0
K Ur 14	1 × 0.10 mg.	0	Trace	0.80	1.00	102.0	143.0*	11.52	+++	0
K Ur 16	1 × 0.10 mg.	0	+	3.00	3.00	74.5	127.0*	17.24	++++	0
K Ur 17	1 × 0.20 mg.	0	+	2.60	4.00	77.5	124.0	13.58	++++	0
K Ur 9	2 × 0.50 mg.	0	+	2.00	3.30	78.5	76.0	10.40	++++	0
K Ur 10	1 × 2.00 mg.	+	+++	2.70	2.20	82.0	112.0	15.04	++++	++
K Ur 12	1 × 2.00 mg.	+	+++	4.60	8.20	62.5	106.0	14.77	+++	++++
K Ur 20	2 × 3.00 mg.	+	++++	....	....	....	.....	14.44	++++	++++
K Ur 21	2 × 3.00 mg.	+	++++	....	....	....	.....	14.07	++++	++++
K Ur 22	2 × 3.00 mg.	+	++++	....	....	....	.....	21.70	++++	++++
K Ur 8	2 × 1.00 mg.	++	+++	3.70	12.80	93.5	85.5	15.35	++++	++
K Ur 13	1 × 2.00 mg.	++	+++	8.70	5.70	83.7	101.0	18.09	+++	+++
K Ur 11	1 × 2.00 mg.	+++	+++	3.80	3.10	79.0	112.0	15.21	++++	+++
K Ur 18	1 × 1.00 mg.	++++	+++	0.30	2.50	89.0	174.0*	14.15	++++	++
K Ur 19	1 × 1.00 mg.	++++	+++	....	3.10	101.0	172.0*	15.63	++++	+++
K Hg 16	1 × 1.00 mg.	0	+	6.83	5.26	....	.....	27.12	++++	+
K Hg 17	1 × 1.00 mg.	0	Trace	3.10	2.66	....	.....	19.05	++++	+
K Hg 20	2 × 1.00 mg.	0	++	2.33	2.80	....	.....	19.11	+++	++
K Hg 21	2 × 1.00 mg.	0	+	4.88	2.75	....	.....	21.45	++++	++
K Hg 22	2 × 1.00 mg.	0	++	4.05	5.72	....	.....	13.56	.....	+
K Hg 23	2 × 1.00 mg.	0	Trace	4.63	13.33	....	.....	18.78	.....	+
K Hg 14	3 × 1.00 mg.	0	.....	1.68	2.14	....	.....	16.90	+++	+++
K Hg 15	3 × 1.00 mg.	0	.....	3.35	3.54	....	.....	9.67	++	+++
K Hg 18	1 × 2.00 mg.	0	.....	4.36	2.88	....	.....	13.61	.....	++
K Hg 19	1 × 2.00 mg.	0	.....	2.98	2.74	....	.....	22.95	+++	+++
K Hg 3	3 × 1.00 mg.	0	+++	....	....	....	.....	6.13	+++	++
K Hg 4	3 × 1.00 mg.	0	+++	....	2.74	....	.....	7.66	+++	++
K Hg 5	1 × 3.00 mg.	0	++++	4.60	....	....	.....	4.28	++	++++
K Hg 7	1 × 3.00 mg.	+	++++	2.92	....	....	.....	8.47	+++	++++
K Hg 9	1 × 3.00 mg.	+	+++	....	....	....	.....	4.99	+++	++++
K Hg 10	1 × 3.00 mg.	+	+++	....	....	....	.....	5.60	+++	++++
K Hg 6	1 × 3.00 mg.	++	++++	3.38	....	....	.....	11.00	+++	++++
K Cr 9	1 × 0.20 mg.	0	Trace	3.40	1.50	80.0	81.0	18.72	+++	0
K Cr 14	1 × 0.50 mg.	0	.....	5.60	3.10	80.5	81.0	17.72	.....	0
K Cr 7	2 × 0.50 mg.	0	++	....	2.90	80.4	126.0*	17.81	++++	0
K Cr 21	2 × 0.50 mg.	0	0	....	....	....	.....	.....	.....	0
K Cr 22	2 × 0.50 mg.	0	++	....	....	....	.....	.. ..	.....	0
K Cr 10	1 × 1.00 mg.	0	++	2.30	2.10	92.0	88.0	11.18	++++	+
K Cr 12	1 × 1.00 mg.	0	.....	2.60	2.80	77.0	60.0	10.92	.....	+
K Cr 15	1 × 1.00 mg.	0	.....	9.50	6.30	75.0	74.0	12.07	+++	+
K Cr 6	2 × 1.00 mg.	0	++	....	4.70	80.7	101.0	12.68	++++	+
K Cr 19	2 × 1.00 mg.	0	+++	....	....	....	.....	.....	.....	++
K Cr 20	2 × 1.00 mg.	0	+++	....	....	....	.....	.....	.....	++
K Cr 11	1 × 2.00 mg.	0	++	1.40	1.50	76.0	74.5	18.86	++++	++
K Cr 17	2 × 3.00 mg.	0	++	....	....	....	.....	7.40	+++	++++
K Cr 8	2 × 1.00 mg.	+	+++	1.40	0.80	94.0	125.0	6.00	+++	++++
K Cr 13	1 × 2.00 mg.	+	.....	2.40	1.50	67.0	80.0	8.00	+++	+++
K Cr 16	2 × 3.00 mg.	+	+++	....	....	....	.....	6.65	++++	+++
K Cr 18	2 × 3.00 mg.	+++	+++	....	....	....	.....	9.60	+++	++++
K Phl 4	2 × 1.00 mg.	..	.....	3.30	3.20	91.0	204.0*	11.00	N	0
K Phl 3	2 × 1.00 mg.	++	Trace	4.30	3.40	56.0	75.5	20.00	N	0
							124.0*			
K Phl 1	2 × 0.50 mg.	++++	0	....	....	78.8	69.0	13.00	N	0
K Phl 2	2 × 0.50 mg.	++++	Trace	2.40	3.30	75.5	64.5	21.70	N	0
K Phl 5	2 × 0.50 mg.	++++	0	1.80	1.60	88.0	138.0*	19.73	N	0

* Blood for determination of sugar was taken from the inferior vena cava while the dog was under ether anesthesia. In the instance of all other dogs blood was drawn from a subcutaneous vein in the leg without anesthesia.

N means normal in amount and in distribution, i. e., in the brush border of the epithelium of the proximal convoluted tubules.

13. Lundsgaard, E.: Biochem. Ztschr. **217**:162, 1930.

14. Kastler, A. O.: J. Biol. Chem. **76**:43, 1928.

Duncan [15] and others have reported hypoglycemia following injections of phlorhizin. This is probably due to depletion of the body's supply of carbohydrate by loss through the urine.

No constant relation was apparent between blood phosphatase and glycosuria when the values for dogs with sugar in the urine were compared with the values for dogs without glycosuria. Poisoning with mercury bichloride produces quantitative changes in the phosphatase of the blood as previously reported by us.[16]

TABLE 2.—*Glycosuria and Blood Sugar After Poisoning with Nephrotoxic Agents*

	Average Value of Blood Sugar, Mg. per 100 Cc.			Dogs with Higher Blood Sugar After Poisoning	
	Before	After	Dogs	No.	Per Cent
$UrO_2(NO_3)_2$					
Glycosuria.....	80.0	103.3	5	4	80
No glycosuria.....	78.0	100.0	2	1	50
$K_2Cr_2O_7$					
Glycosuria.....	80.5	102.5	2	2	100
No glycosuria.....	80.2	79.9	7	3	43
Phlorhizin					
Glycosuria.....	80.7	69.7	3	0	0
No glycosuria.....	91.0	....	1		

The values of blood phosphatase and their relation to glycosuria in this series of dogs are shown in table 3. After the administration of uranyl nitrate, the average value of blood phosphatase in dogs with and without glycosuria was increased, but only 50 and 60 per cent, respectively, of these dogs had higher phosphatase after poisoning with this substance. After poisoning with mercury bichloride one half of the dogs without glycosuria had higher blood phosphatase than before the poison was administered. Potassium dichromate ap-

TABLE 3.—*Glycosuria and Alkaline Blood Phosphatase*

	Average Value of Blood Phosphatase, Bodansky Units per 100 Cc.			Dogs with Higher Blood Phosphatase After Poisoning	
	Before	After	Dogs	No.	Per Cent
$UrO_2(NO_3)_2$					
Glycosuria.....	3.97	5.37	6	3	50.0
No glycosuria...	2.28	3.26	5	3	60.0
$HgCl_2$					
Glycosuria.....	3.15	....	2	?	
No glycosuria...	3.89	4.23	10	5	50.0
$K_2Cr_2O_7$					
Glycosuria.....	1.90	1.15	3	0	0.0
No glycosuria...	4.13	3.11	6	2	33.3
Phlorhizin					
Glycosuria.....	2.83	2.77	3	1	33.3
No glycosuria...	3.30	3.20	1	0	0.0

peared to lower blood phosphatase. In 4 dogs blood phosphatase was slightly reduced after injection of phlorhizin. Anderson and Squires,[17] who used larger doses than were employed in our experiments, found that phlorhizin-induced diabetes was accompanied by a decided increase in serum phosphatase.

The relation of renal alkaline phosphatase (stated in Bodansky units) to glycosuria is shown in table 4.

15. Duncan, G. G.: Diseases of Metabolism, Philadelphia, W. B. Saunders Company, 1942, pp. 24 and 694.

16. Hepler, O. E.; Gurley, H., and Simonds, J. P.: Arch. Path. **39**:133, 1945.

17. Anderson, R. K., and Squires, R. B.: J. Biol. Chem. **124**:71, 1938

The relation of renal phosphatase to glycosuria revealed by Gomori's method in these dogs can be seen in table 1. Among the dogs poisoned with uranyl nitrate, the mean value of renal phosphatase was lower in those without glycosuria but was equal to or greater than the normal mean of 10.72 ± 0.33 in all dogs of both groups.[16] The difference was much greater in the dogs poisoned with mercury bichloride and potassium dichromate. Not only was the phosphatase lower in the dogs with glycosuria than in those without sugar in the urine, but it was 30 per cent below the normal mean of the controls in this series.[16] Among 13 dogs poisoned with mercury bichloride but without glycosuria, renal phosphatase was less than the normal mean in 4, while of the 4 with sugar in the urine, 3 were well below and 1 was within the limits of error of the normal mean. Renal phosphatase in the potassium dichromate dogs averaged 29 per cent below the normal mean in all animals with glycosuria and in only 1 dog without glycosuria. Sections of the kidneys of these dogs stained by Gomori's method [2] revealed phosphatase in the epithelium of the proximal convoluted tubules both in those with and in those without glycosuria. Among the dogs given phlorhizin renal phosphatase was lower in the dog whose urine was not tested for sugar than in the 4 dogs whose urine contained sugar, but it was above the normal mean in all. In sections of the

TABLE 4.—*Glycosuria and Alkaline Renal Phosphatase*

	Dogs	Mean Value of Phosphatase, Bodansky Units	Standard Deviation
$UrO_2(NO_3)_2$			
Glycosuria.....	10	16.15 ± 0.45	2.12 ± 0.32
No glycosuria...	5	13.76 ± 0.84	2.80 ± 0.32
$HgCl_2$			
Glycosuria.....	4	7.51 ± 0.82	2.44 ± 0.57
No glycosuria...	13	15.41 ± 1.25	6.68 ± 0.87
$K_2Cr_2O_7$			
Glycosuria.....	4	7.56 ± 0.46	1.38 ± 0.33
No glycosuria...	9	13.80 ± 0.89	3.96 ± 0.63
Phlorhizin			
Glycosuria.....	4	18.58	
No glycosuria...	1	11.00	

kidneys of the 5 phlorhizin dogs stained by Gomori's method, phosphatase was present in normal amount and in normal distribution.

For 12 dogs of this group blood urea was determined along with sugar in the blood and the urine. There was no constant relation between these two excreted substances. This is in harmony with the observation of Wallace and Myers.[18] Glycosuria was not present in any of the 4 dogs with a blood urea concentration less than 20 mg. per hundred cubic centimeters. However, 1 dog poisoned with potassium dichromate with only 23.4 mg. of urea per hundred cubic centimeters of blood had a high content of sugar in the urine, while another with 61.8 mg. per hundred cubic centimeters did not have glycosuria. In the other 6 dogs of this group the blood urea ranged from 78 mg. per hundred cubic centimeters upward, and all had glycosuria.

SUMMARY AND RESULTS

The incidence and the extent of glycosuria in dogs poisoned with chemical agents were compared with other factors determined simultaneously. Some degree of correlation was found

18. Wallace, G. B., and Myers, H. B.: J. Pharmacol. & Exper. Therap. **5**:511, 1913-1914.

between glycosuria and (1) tubular necrosis, (2) albuminuria and (3) the chemical agent and its dose. No definite correlation was observed between glycosuria and blood sugar, blood urea, blood phosphatase and renal phosphatase.

All the dogs that had sugar in the urine except those given phlorhizin also had marked albuminuria and extensive necrosis of the tubular epithelium. But the converse was not true. The most constant and definite correlation was found between glycosuria and the chemical agent and the dose used. Uranyl nitrate was the most potent of the three inorganic agents in producing glycosuria, for each dog that received 1 mg. or more per hundred cubic centimeters of blood was found to have sugar in its urine. Two milligrams of potassium dichromate per hundred cubic centimeters of blood were required to produce glycosuria, and this dose was effective in only 44.4 per cent of the dogs. Three milligrams of mercury bichloride produced glycosuria in only 80 per cent of the animals receiving this dose.

Uranyl nitrate appeared to produce a moderate increase in blood sugar in dogs with and without glycosuria; potassium dichromate produced a moderate increase in blood sugar in the dogs with glycosuria but not in those in which the urine was free from sugar; phlorhizin appeared to lower the blood sugar slightly. Uranyl nitrate tended to increase the blood phosphatase, while potassium dichromate and phlorhizin decreased it slightly. In the dogs poisoned with uranyl nitrate, renal alkaline phosphatase was above the normal mean both in those with and in those without glycosuria but was higher in those without sugar in the urine. Among the dogs poisoned with mercury bichloride and potassium dichromate, the mean renal phosphatase was markedly reduced in those with glycosuria and was increased in those without glycosuria. Renal phosphatase was markedly increased in 4 phlorhizinized dogs with glycosuria, while in 1 dog for which we have no data concerning sugar in the urine renal phosphatase was within the limits of error of the normal mean.

COMMENT

Previous investigators have quite generally concluded (1) that the glycosuria which follows poisoning with chemical agents, particularly with uranium, which has been most extensively studied in this connection, is of renal origin and due to damage of the renal tubules and (2) that it is not related to blood sugar levels.[9] We agree with the first conclusion but we are not in full accord with the second. There also remain for discussion (1) the differences in power to produce glycosuria possessed by the different chemical agents used in these experiments, (2) the mechanism of the glycosuria and (3) its relation to renal phosphatase.

An observation of Shannon, Farber and Troast[19] is pertinent to the relation between blood sugar levels and glycosuria under the conditions of these experiments. When glucose reabsorption is studied in the normal dog with progressive increments in the plasma arterial concentration of glucose, the plasma level at which frank glycuresis appears is essentially the same as that at which glucose *Tm*, or complete saturation of all the nephrons, is reached. It is likely that there is a variation in the size of the glomeruli and in the length of the renal tubules in any normal animal. These factors are not independent but are coordinated parts of the structural-functional relationships of the nephron as a whole. The higher the filtration rate of any glomerulus in relation to the ability of its tubule to reabsorb glucose, the lower the plasma glucose concentration which is necessary to saturate the reabsorptive capacity of the tubule.

The chemical agents in the dosages employed in these experiments did not produce visible injury in the glomeruli but, except for phlorhizin, they did damage the tubular epithelium in those dogs that had glycosuria. These conditions upset the balance between the correlated capacities of the glomeruli and the tubules—the one to produce filtrate and the other to reabsorb glucose from the filtrate. The reabsorptive capacity of the tubules thus damaged would be completely saturated at a lower plasma glucose concentration than that of a normal animal. Any rise in the plasma glucose level from any cause would therefore make glycosuria a necessary consequence and increase its degree. A normal or even a lowered plasma glucose concentration could, under such conditions, be accompanied by glycosuria.

In view of the statements in the two immediately preceding paragraphs the mechanism of glycosuria in the dogs poisoned with the inorganic substances used in these experiments is relatively simple. The damage to the epithelium of the tubules reduces the glucose *Tm* to such an extent that a normal or even a reduced glucose plasma concentration is in excess of that required to saturate all the nephrons. In diabetes mellitus the high plasma glucose oversatu-

19. (*a*) Shannon, J. S.; Farber, S., and Troast, L.: Am. J. Physiol. **133**:752, 1941. (*b*) Mirsky, I. A., and Nelson, N.: Arch. Int. Med. **71**:827, 1943.

rates the normal *Tm*,[19b] in poisoning with these chemical agents the normal plasma glucose more than saturates the reduced *Tm*. Glycosuria results in both cases.

It is difficult to explain the difference in power of uranyl nitrate, mercury bichloride and potassium dichromate to produce glycosuria. The difference in dosage required has been mentioned. Glycosuria was clearly dependent on necrosis, but that necrosis was not the sole factor involved is evident on reference to table 1. In that table necrosis is evaluated as 1 plus to 4 plus. Necrosis graded 1 plus involved only an occasional tubule and was limited to the aglomerular, subcapsular zone and to the labyrinth in dogs poisoned with potassium dichromate and to the terminal, straight portion of an occasional proximal convoluted tubule along the margins of the labyrinth in dogs poisoned with mercury bichloride and uranyl nitrate. Necrosis of 4 plus grade involved the greater part of all the tubules. Two plus and 3 plus grades lay between these extremes.

Among the glycosuric dogs poisoned with uranyl nitrate, 3 had necrosis of only 2 plus grade, and the lowest sugar levels were in the dogs with 4 plus necrosis. On the other hand, all the dogs poisoned with mercury bichloride and potassium dichromate that became glycosuric had 3 and 4 plus necrosis. It does not seem possible, therefore, that the low levels of urinary sugar in dogs poisoned with uranyl nitrate could have been due simply to sugar's having diffused through the necrotic material in the tubules into the peritubular capillaries, although the osmotic pressure of the plasma proteins in these capillaries is higher than in any other groups of capillaries in the body because of the loss of water in the glomeruli.

A comparison of the differential effects of these three chemical agents on blood sugar and on blood and renal phosphatase does not warrant the conclusion that any one of these factors was concerned in the difference in power to produce glycosuria. Differences in the location of the tubular damage with the doses employed in these experiments do not furnish a satisfactory explanation; for uranyl nitrate and mercury bichloride affect the same portion of the proximal convoluted tubule, and yet the former is clearly much more potent in the production of glycosuria than is the latter, which causes a higher degree of calcification.[20] At present, therefore, we have no explanation for the fact that smaller doses of uranyl nitrate with lower grades of necrosis produce glycosuria with greater constancy and to a more marked degree than do mercury bichloride and potassium dichromate.

In a previous paper[16] we suggested the possibility—and only the possibility—that the mere presence of phosphatase in the brush border of the proximal convoluted tubules is not necessarily proof that it takes part in the functioning of these tubules. Its presence *might* be due to an abortive attempt to reabsorb from the glomerular filtrate a molecule too large for these cells to transmit through their cytoplasm. We found no evidence that this enzyme is concerned with the deposition of calcium in the kidneys of dogs poisoned with the nephrotoxic substances uranyl nitrate, mercury bichloride and potassium dichromate. The experiments here reported show that glycosuria can occur in dogs whose kidneys contain either an abundance of phosphatase (uranyl nitrate and phlorhizin) or a reduced amount of this enzyme (mercury bichloride and potassium dichromate). There is a much closer correlation between glycosuria and necrosis of the proximal convoluted tubules than between glycosuria and the quantity of phosphatase present in the kidneys. As pointed out in previous papers,[21] phosphatase may be abundant in kidneys with extensive necrosis. The large amount of phosphatase in these cases could be due to adsorption of the enzyme by the granular necrotic material in the tubular lumens.

We have been unable to find any constant or provable correlation between alkaline phosphatase activity in the kidneys and any normal or pathologic processes occurring in these organs. The mechanism of the action of alkaline renal phosphatase in the performance of its alleged function is still a hypothesis based on one or more postulates.[22]

During the past decade or more much of the literature which has dealt with the mechanism of reabsorption of glucose by the renal tubule has been concerned with the phosphorylation of this sugar. Martland and Robison,[23] Kay[24] and Lundsgaard[25] expressed the belief that phosphatase is a reversible enzyme capable both of synthesizing and of dephosphorylating hexosephosphate. This was later disputed by Lunds-

20. (*a*) Hepler, O. E., and Simonds, J. P.: Arch. Path. **40**:37, 1945. (*b*) Hepler, O. E.; Gurley, H., and Simonds, J. P.: Proc. Soc. Exper. Biol. & Med. **44**:221, 1940.

21. Footnotes 16 and 20.

22. Kalckar, H.: Chem. Rev. **28**:71, 1941.

23. Martland, M., and Robison, R.: Biochem. J. **21**:665, 1927.

24. Kay, H. D.: Biochem. J. **22**:855, 1928.

25. Lundsgaard, E.: Biochem. Ztschr. **264**:209 and 221, 1933.

gaard himself,[26] by Kalckar [27] and by Lipmann.[28] The difficulties associated with the attempt to relate phosphorylation to a synthesizing action of phosphatase have been recently summarized by Kritzler and Gutman.[29] Kalckar [22] and Colowick and co-workers [30] have presented evidence that phosphorylation of glucose occurs as a result of a specific enzyme system, renal phosphorylase, which constitutes a part of the specific cellular mechanisms postulated to explain the rapid transport of glucose from the lumens of the proximal convoluted tubules to the blood stream. Wilmer,[7] using this concept, has presented a theoretic scheme according to which phosphorylase synthesizes hexose phosphate in the tubular epithelial cells; phosphatase then breaks down this compound and liberates the glucose, which is transmitted through the cells to the tissue spaces and thence to the blood in the intertubular capillaries.

Wilmer's interesting theory presents certain mechanical difficulties. Phosphatase is known to be limited to the brush border of the epithelium of the proximal convoluted tubules. The location of the phosphorylase in these cells is not known. Since phosphorylase, according to this theory, must act on glucose first, it should theoretically, be nearer the sugar to be absorbed from the tubular lumens than is phosphatase. This can hardly be true. If the phosphorylating enzyme is also in the brush border, it might be possible for two opposing actions to take place within such a limited space, but it certainly adds difficulties to the acceptance of the hypothesis. If phosphorylase is distributed throughout the cell, it seems likely that it would rephosphorylate glucose that had been dephosphorylated by phosphatase nearer the lumen of the tubule. In our experiments the nephrotoxic substances caused damage and necrosis of the tubular epithelium and diffusion of phosphatase throughout the cell. This would obviously disturb the spatial relations of the two enzymes and interfere markedly with whatever combined function they may perform.

Although many authors refer to the presence of phosphorylase in the kidneys, there appear to have been few quantitative determinations of the content. Shapiro and Wertheimer,[31] however, have recently made such determinations and concluded that organs with a highly active glycogen metabolism, e. g., skeletal and cardiac muscle and liver, have much more active phosphorylase than do organs such as the lungs, the skin, the testes, the intestines, the spleen, adipose tissue and the placenta, with less active glycogen metabolism. The kidneys and the brain occupy an intermediate position both in phosphorylase activity and in glycogen metabolism. They found that the kidneys converted glucose-1-phosphate into only about 50 per cent of the theoretic maximum polysaccharide. Glycogenolysis in the kidneys amounted to 0.75, compared with about 2.0 for that of skeletal and cardiac muscle and liver.

If phosphatase does take part in renal function it would seem to be a logical hypothesis that it must be concerned with the absorption of glucose from the glomerular filtrate. However, we have found no correlation and no constant relationship between alkaline renal phosphatase and the glycosuria which accompanied poisoning with uranyl nitrate, mercury bichloride and potassium dichromate. But the absence of any such relationship under the conditions of these experiments does not exclude the possibility that this enzyme may function in normal kidneys after the manner described by Wilmer [7] or in some other way. These nephrotoxic substances in adequate doses produce massive necrosis of the tubular epithelium, but active phosphatase is still present in the necrotic material.[16] We do not know what effect these agents have on phosphorylase, the action of which must, theoretically, precede that of phosphatase.

Various chemical substances have been found either to accelerate or to inhibit the action of phosphatase. These were reviewed in a previous paper.[16] There is little available information concerning the effects of chemical agents on phosphorylase. It is said to be inhibited by phlorhizin,[25] by lack of oxygen,[32] by a cyanide,[32] by iodoacetic acid,[33] by traces of copper,[34] by ammonium sulfate [35] and by sodium beta glycero-

26. Lundsgaard, E.: Skandinav. Arch. f. Physiol. **72**:265, 1935.

27. Kalckar, H.: Enzymologia **2**:47, 1937.

28. Lipmann, P., in Nord, F. F., and Werkman, C. H.: Advances in Enzymology, New York, Interscience Publishers, Inc., 1941, vol. 1, p. 99.

29. Kritzler, R. A., and Gutman, A. B.: Am. J. Physiol. **134**:94, 1941.

30. (*a*) Colowick, S. P.; Welsh, M. S., and Cori, C. F.: J. Biol. Chem. **133**:359, 1940. (*b*) Colowick, S. P.; Kalckar, H., and Cori, C. F.: ibid. **137**:243, 1941.

31. Shapiro, B., and Wertheimer, E.: Biochem. J. **37**:397, 1943.

32. Footnotes 22 and 27.

33. Wilbrandt, W., and Laszt, L.: Biochem. Ztschr. **259**:308, 1933.

34. Cori, G. T., and Cori, C. F.: J. Biol. Chem. **133**:733, 1940.

35. (*a*) Cori, C. F.; Cori, G. T., and Green, A. A.: J. Biol. Chem. **151**:39, 1943. (*b*) Evans, E. A., Jr., in Luck, J. M., and Smith, J. H. C.: Annual Review of Biochemistry, Stanford University, Calif., Annual Reviews, Inc., 1944, vol. 13, p. 187.

phosphate.[35b] Its action is said to be accelerated by reducing substances, such as glutathione, and by a fluoride.[22] Reducing agents act directly on the phosphorylase. The fluoride inhibits alkaline phosphatase and thus blocks dephosphorylation. This permits the accumulation of hexose–phosphoric ester, which would otherwise quickly disappear. The fluoride is thus only an indirect accelerator. Neither our experiments nor any reports discovered in the literature furnish information concerning the effects of the inorganic nephrotoxic agents used by us on this enzyme. Until these effects are known it will not be possible to interpret our results accurately.

The concept that phlorhizin produces its effects on the tubular absorption of dextrose by blocking phosphorylation appears to have originated with Lundsgaard.[25] In 1933 he reported that in digestion experiments with muscle *brei* (broth) and with muscle, yeast and kidney extracts phlorhizin in fiftieth molar to two-hundredth molar concentrations inhibited the processes of esterification and dephosphorylation. He then believed that, because of the absorption of water in the proximal convoluted tubules, a concentration of phlorhizin might be attained in the kidneys capable of inhibiting the phosphorylation of glucose. In later experiments (1935) he concluded [26] that the concentration of phlorhizin in the kidneys necessary to induce maximal action (glycosuria) was less than one fifth of that required to produce the inhibiting effects observed in his earlier experiments. He admitted that from these experiments no support can be gained for the assumption that the action of phlorhizin on the kidneys is due to the earlier demonstrated effect of the poison on esterification in vitro. From their own experiments, Walker and Hudson [36] came to a similar conclusion.

Most investigations on the action of phlorhizin have been based on the use of relatively large doses of the glucoside. Lundsgaard [25] used from 2.36 (two-hundreth molar) mg. to 9.44 (fiftieth molar) mg. Walker and Hudson [36] used doses that were never less than 30 mg. per kilogram, and in the majority of their experiments the dose was from 150 to 550 mg. per kilogram. Throughout the experiments reported in this series of papers we intentionally used very small doses of each of the poisons employed. Our dogs were given 0.5 and 1.0 mg. per hundred cubic centimeters of blood intravenously, which is close to the same amount per kilogram of body weight, and yet these doses produced glycosuria.

36. Walker, A. M., and Hudson, C. L.: Am. J. Physiol. **118**:130, 1937.

Kalckar,[37] however, has described active oxidative phosphorylation in extracts of kidney which was inhibited by phlorhizin. He thus revived Lundsgaard's [25] original hypothesis. Much of the more recent work has followed the course set by Kalckar.

The problem of the relation of phlorhizin to phosphorylation is not as simple as the hypothesis of Wilmer [7] and others suggests. If phlorhizin interferes with the absorption of glucose solely by blocking its phosphorylation, it should not interfere with the absorption of other substances that cannot be phosphorylated or with other functions of the kidneys. This is not the case. Ellinger and Lambrechts,[38] using frogs, found that phlorhizin interfered with reabsorption from the proximal convoluted tubules of rhodamine B. S., and of fluorescein. The former is capable of phosphorylation, while the latter cannot be phosphorylated. White [39] found that phlorhizin lowers the transfer of diodrast as much as 60 per cent. This substance is excreted but is not absorbed by the tubular epithelium. This observation suggests that the effect of phlorhizin on the kidneys is more widespread than that of merely interfering with phosphorylation. Kalckar [40] has stated that this glucoside is not a specific inhibitor of phosphorylation. Lambrechts [41] has also stated that phlorhizin acts not by an inhibition of phosphorylation but by a toxic action on the cells. London and Kotschneff [42] presented evidence that phlorhizin involves organs in addition to the kidneys and causes a toxic disturbance more widespread than glycosuria. Furthermore, Shannon [43] has stated that preliminary experiments on otherwise normal dogs suggest that phlorhizin may inhibit the active tubular reabsorption of glucose by entering into competition with glucose for the transport mechanism and by displacing glucose from it in much the same way in which glucose can exclude xylose.

The most fundamental criticism of the concept that phlorhizin produces glycosuria by blocking phosphorylation has been presented by Weiss-

37. Kalckar, H.: Skandinav. Arch. f. Physiol. **77**: 46, 1937.

38. Ellinger, P., and Lambrechts, A.: J. Physiol. **89**:30P, 1937.

39. White, H. L.: Am. J. Physiol. **130**:582, 1940.

40. Kalckar, H.: Nature, London **136**:872, 1935.

41. Lambrechts, A.: Arch. internat. de physiol. (supp.) **44**:1, 1937.

42. London, E. S., and Kotschneff, N.: Arch. f. exper. Path. u. Pharmakol. **178**:700, 1935.

43. Shannon, J. A., in Luck, J. M.: Annual Review of Physiology, Stanford University, Calif., Annual Reviews, Inc., 1942, vol. 4, p. 297.

berger.[44] In her studies of phosphorylation she used "tagged" radioactive phosphorus. By ordinary analytic methods, only the amounts and the changes in the amounts of compounds in the tissues are observable. By the use of isotopes it is possible to demonstrate both the rates and the changes in the rates at which chemical reactions proceed in the body. Since the alleged inhibition of phosphorylation by phlorhizin need not be accompanied by change in the absolute or the relative amounts of the phosphorus compounds in the tissues concerned but only by change in the rate of phosphorus turnover, radioactive phosphorus furnishes a useful tool for the examination of this theory. A comparison of phosphorus turnover after the administration of radioactive phosphorus revealed no differences in normal and phlorhizinized rats in the rate of incorporation of the radioactive phosphorus into the kidney, the intestine, the blood or the liver or into the renal, the hepatic or the intestinal phospholipds. Neither was there any difference in the excretion of radioactive phosphorus in the two groups of rats, although the phlorhizinized animals showed marked diuresis and glycosuria. Contrary to the hypothesis that the biologic action of phlorhizin is due to retardation of phosphorylation, Weissberger concluded that phlorhizin does not inhibit processes of phosphorylation in the intact animal and that it must produce its effect by some other mechanism.

Phlorhizin produces glycosuria without altering the amount or the location of the phosphatase, without visibly injuring the cells of the tubules and without disrupting the structural pattern of the kidneys. The inorganic chemical agents used in these experiments produce glycosuria only (1) after markedly altering the distribution, but without necessarily reducing the amount, of phosphatase in the kidneys, (2) after causing extensive necrosis of the tubular epithelium and (3) after completely disrupting the structural pattern of a considerable portion of the proximal convoluted tubules. The mechanism of phlorhizin glycosuria is not, therefore, comparable to that of the glycosuria induced by these inorganic poisons.

44. Weissberger, L. H.: J. Biol. Chem. **139**:543, 1941.

Shannon[45] has stated that "there is present in the tubular epithelium of all species a limited amount of a stable cellular element whose specific function it is to combine temporarily with the solute transferred." In the various attempts to explain the glycosuria caused by certain chemical agents, one factor has not been given adequate attention. Each organ performs its functions through the medium of its own peculiar structural pattern. In dogs poisoned with the nephrotoxic substances used in these experiments, not only were the epithelial cells of the tubules killed but the structural pattern of the tubules was disrupted. Under such conditions, although the enzymes or the "stable cellular element" of Shannon,[45] on which a particular process depends, may be present and active in normal, or even in excessive, amounts they cannot function because of alterations in the anatomic structure on which their action depends. This principle would apply whatever may be the mechanism of the reabsorption of sugar by the proximal convoluted tubules.

SUMMARY

Glycosuria was produced in dogs with uranyl nitrate, mercury bichloride, potassium dichromate and phlorhizin.

A marked difference was observed in the power of the three inorganic chemical agents to produce glycosuria. Uranyl nitrate was the most potent.

Some degree of correlation was found between the glycosuria and (*a*) the necrosis of the tubular epithelium, (*b*) the albuminuria and (*c*) the chemical agent and its dose.

No definite or constant correlation was observed between the degree of glycosuria and the levels of sugar and urea in the blood or the amount of phosphatase in the blood or in the cortex of the kidney.

Our results shed no light on the possible role of phosphatase in the absorption of glucose by the kidneys because no information is available concerning the effect of the inorganic chemical agents used on phosphorylase.

Certain fundamental differences are presented in the conditions and in the mechanism of the glycosuria induced by phlorhizin and that caused by inorganic poisons.

45. Shannon, J. A.: Physiol. Rev. **19**:63, 1939.

LEUKOPENIA AND INFLAMMATION

The Presence of a Leukopenic Factor in Inflammatory Exudates

VALY MENKIN, M.D.
DURHAM, N. C.

A NUMBER of inflammatory conditions are accompanied by a fall in the number of leukocytes in the blood stream, a so-called state of leukopenia. Lawrence [1] recently discussed the varying conditions which may bring about this picture. These conditions may involve inhibition of the maturation of leukocytes, active elimination of these cells or possibly their destruction. Fitz-Hugh and Krumbhaar [2] have considered agranulocytosis, first described by Schultz,[3] as the result of an arrest of the development of leukocytic elements. In its severe state the disease involves lymphoid cells as well as granulocytes. For this reason it is referred to as pernicious leukopenia.[2] Profound leukopenia referable to a virus infection has been recently reported to occur in cats.[4] It is interesting to note on close scrutiny the frequency with which some infection accompanies an agranulocytic process.[2] Dameshek [5] and Sturgis [6] recently reviewed the literature on the subject of leukopenia. Sturgis [6] expressed the belief that agranulocytic conditions occur frequently as a result of drug sensitizations. He directed attention to such drugs as aminopyrine [7] and gold salts. Sturgis [6] expressed the view that the mechanism is a delay in the maturation of granulocytes in the bone marrow. With reduction in number of leukocytes, susceptibility to infection is increased.[6] Bacterial invasion in various parts of the body results

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1. Lawrence, J. S.: J. A. M. A. **116**:478, 1941.

2. Fitz-Hugh, T., and Krumbhaar, E. B.: Am. J. M. Sc. **183**:104, 1932.

3. Schultz, W.: Deutsche med. Wchnschr. **48**:1495, 1922.

4. Lawrence, J. S.; Syverton, J. T.; Shaw, J. S., and Smith, F. P.: Am. J. Path. **16**:333, 1940. Hammon, W D., and Enders, J. F.: J. Exper. Med. **69**:327, 1939.

5. Dameshek, W., in Christian, H. A.: Oxford Medicine, New York, Oxford University Press, 1944, vol. 3.

6. Sturgis, C. C.: Clinics **1**:492, 1942.

7. Sturgis, C. C., and Isaacs, R.: Tr. A. Am. Physicians **46**:328, 1934.

in cellular damage and necrosis, which are characteristically found in agranulocytosis.[6] In my mind it is questionable whether the leukopenia always precedes the infection or whether the severe inflammatory condition per se may not be a predisposing factor in favoring the development of the leukopenic phase. The mechanism of the persistent leukopenia encountered in various inflammatory processes—for instance, typhoid, influenza or tuberculosis—has not been satisfactorily explained.

I have demonstrated the presence of an injurious factor located in, or at least closely associated with, the englobulin fraction of inflammatory exudate.[8] This substance has been termed necrosin. It has been identified not only in canine exudates but also in human exudative material.[8] Smith and Smith [9] have recently confirmed the observation that this substance is present in canine exudates. They [9] have pointed out the presence of a closely similar toxic material in menstrual blood. This is not wholly surprising, for such material contains disintegrated cellular products from the endometrium combined with elements from the blood. Menstrual blood can perhaps be considered as a sort of modified physiologic exudative material, provided exudate, as such, is defined as the products of cellular injury combined with a varying amount of hematic elements. Menstrual blood is therefore viewed by me as primarily a hemorrhagic type of a somewhat modified exudative material. The additional rupture of endometrial vascular structures doubtless is responsible for the hemorrhagic appearance of this exudate. As pointed out in an earlier publication, necrosin shows proteolytic activity which seems to be capable of being held in abeyance by an antiprotease.[10] The observation of this enzymatic property, which may yet prove to be independent of the toxic substance in necrosin, has also been confirmed.[9]

Recent studies indicate the more frequent but not invariable occurrence of necrosin in exudates derived from inflammations at an acid p_H.[11] It must be pointed out in this connection, however, that necrosin has at times been obtained from exudates at an alkaline p_H. But the material seems to be more frequently present in acid exudates.[11]

The whole euglobulin fraction of exudate not only induces marked cutaneous injury in the rabbit but causes definite fever and pronounced leukopenia in dogs.[12] Subsequent investigations have revealed that the pyrogenic property of the whole euglobulin fraction is referable not to the thermolabile necrosin but to a different but closely associated thermo-

8. Menkin, V.: Arch. Path. **36**:269, 1943.

9. Smith, O. W., and Smith, G. V. S.: Proc. Soc. Exper. Biol. & Med. **59**:116, 1945.

10. Menkin, V.: Am. J. M. Sc. **208**:290, 1944; Science **100**:337, 1944.

11. Menkin, V.: Federation Proc. **4**:149, 1945.

12. Menkin, V.: Proc. Soc. Exper. Biol. & Med. **54**:184, 1943; footnote 8.

stable substance, termed by me pyrexin.[13] The thermostability of pyrexin, on the one hand, and the unimpaired toxicity of purified necrosin in distilled water, on the other, nullifies the contention that pyrexin is referable to the technical procedure adopted in its preparation.[9]

The present communication indicates that in inflammatory exudates there is a leukopenic factor which is not one of the biologic properties of purified necrosin.[14] It is most often associated with pyrexin; yet it can readily be dissociated, at least to a large extent, from this pyrogenic factor. The presence of such a leukopenic factor in inflammatory exudates in large part explains, perhaps, the leukopenia accompanying numerous inflammatory processes. The leukocytosis-promoting factor (abbreviated as the LPF) present in exudates may well mask the ultimate effect of the leukopenic factor.[15] In brief, the final blood picture accompanying an acute inflammatory process may to a large extent depend on the relative concentration of either the leukocytosis-promoting factor or the leukopenic factor now under discussion, both factors being produced at the site of an acute inflammation.

EXPERIMENTAL STUDY

To obtain exudative material, 1.5 cc. of turpentine was injected into the canine chest cavity, as previously described.[15] It was found that the leukopenic factor is more likely to be recovered from exudates that are at an acid p_H. For this reason the material was recovered from a cavity with a severe pleural inflammation of several days' duration, with its content at an acid reaction, or else, to hasten the process of acidification, the irritant was reinjected into the pleural cavity. This secondary treatment often tended to induce leukopenia in the circulation besides rendering the animal considerably ill. The leukopenic factor, as a rule, was recovered from such exudative type of material.

The whole exudate in amounts ranging from about 5 to 20 cc. was injected intracardially into dogs, and the leukocyte counts were taken approximately every hour for a period of five to six hours from samples of blood obtained by nicking the ear. Under such circumstances it is usually observed that there is an initial drop in the number of circulating leukocytes during the first two hours after the intravascular injection of the material. This is illustrated in chart 1. After an interval the number of leukocytes rises and definite leukocytosis is then in evidence. This is probably referable primarily to the presence of the leukocytosis-promoting factor in the exudate. The data of the various experiments are assembled in table 1. It is quite clear that within the first two hours after the intravascular injection of exudative material there is an appreciable reduction in the number of circulating leukocytes. From an average basal level of 11,672 white blood cells per cubic millimeter there is an average fall of 3,778 in the first two hours following injections of exudate in otherwise normal dogs.

13. Menkin, V.: Arch. Path. **39**:28, 1945.

14. Menkin, V.: Science, to be published.

15. Menkin, V.: Arch. Path. **30**:363, 1940; Dynamics of Inflammation, New York, The Macmillan Company, 1940.

This appreciable lowering of the number of circulating leukocytes in eight experiments indicates the probable presence of a leukopenic factor in such exudative material.

This leukopenic factor is not present in normal blood serum (table 2 and chart 1). In four experiments in which normal blood serum was introduced into the circulation of dogs, it was noted that the number of circulating leukocytes failed to be altered appreciably within several hours after the injections. These

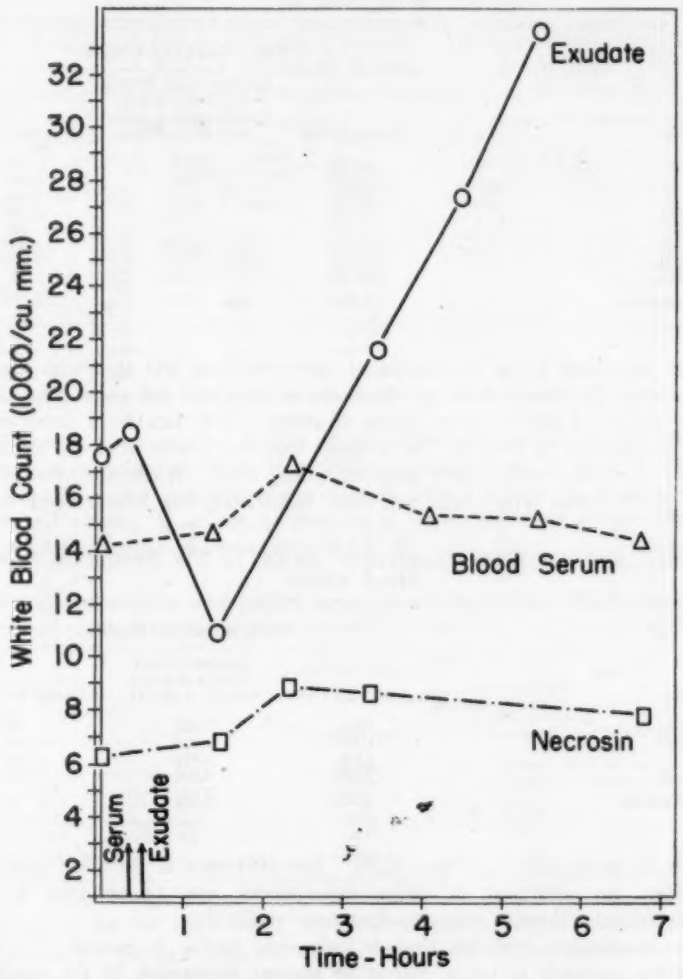

Chart 1.—Effect of necrosin, exudate and serum on the white blood cell count. First, the presence of a leukopenic factor in whole exudate is demonstrated (0——0——0; dog 23-D). The subsequent leukocytosis shown in this curve is likely referable in part at least to the presence of the leukocytosis-promoting factor in the exudative material. That the leukopenic factor is not present in either serum (dog 4-27) or necrosin (dog 29-D) is shown in the two lower curves.

observations clearly indicate that a leukopenic factor is present in exudate but not in normal nonhemolyzed blood serum.

A series of observations was made on dogs on days on which they were not given injections of any material. Some of these animals were the same ones that had been given injections of various fractions of exudates on other days. These experiments were made with the purpose of establishing the normal fluc-

TABLE 1.—*Evidence That a Leukopenic Factor Is Present in Whole Exudate at an Acid p_H*

	White Blood Cell Counts		
Dog	Basal Count	Initial Count Within 1 to 2 Hr. After Intravascular Injection of Exudate	Drop in Count
4-27	14,950	9,850	5,100
4-14	10,900	7,400	3,500
4-14	11,925	8,325	3,600
4-25	9,175	3,750	5,425
4-17	7,875	7,575	300
18-D	9,350	8,300	1,050
18-D	10,700	6,950	3,750
23-D	18,500	11,000	7,500
Average	11,672	7,894	3,778

tuation in the white blood cell counts of untreated dogs and also with the view of determining the lowest level to which the white blood cell count usually falls within a period of six to seven hours of study. The data from these experiments are collected in table 3. It is clear that the lowest level of circulating leukocytes is not markedly lower than the initial level. While the average basal level is 10,222 white blood cells per cubic millimeter, the lowest level reached

TABLE 2.—*Evidence That a Leukopenic Factor Is Not Present in Normal Blood Serum*

	White Blood Cell Counts		
Dog	Basal Cell Count	Lowest Count Within Several Hours of Study	Change in Count
4-14	7,575	7,525	— 50
4-27	14,150	14,300	+ 150
4-19	5,855	5,575	— 280
4-29	7,100	7,350	+ 250
Average	8,670	8,688	+ 18

within six to seven hours averages 9,225. The difference is therefore clearly not appreciable. As indicated in three experiments, the temperature in such untreated animals likewise scarcely fluctuates (table 3).

These observations indicate that a leukopenic factor is present in exudate and that the evidence is not a matter of normal fluctuation in the number of circulating leukocytes. Furthermore, the factor is more likely to be recovered, though not exclusively, from exudate which is at an acid p_H at the time it is withdrawn from the thoracic cavity. The factor is not present in blood serum, as indicated in the foregoing paragraph.

A search was undertaken in an endeavor to determine whether the leukopenic factor can be isolated from inflammatory exudates. In an earlier study [3] it was

shown that the whole euglobulin fraction of exudate, besides being capable of inducing marked injury of tissue, has pyrogenic and leukopenic properties. Further purification of the euglobulin fraction has indicated that the toxic property is referable to the euglobulin part, and this factor has been termed necrosin, while the pyrogenic effect has been ascribed to another substance associated with the euglobulin fraction and accordingly named pyrexin.[13] Is the leukopenic property of the whole euglobulin fraction[8] associated with necrosin or with pyrexin?

TABLE 3.—*Lowest Level to Which Circulating Leukocytes Fell During a Study of Normal Variations in Their Number in Days*

	White Blood Cell Counts			Change in Temperature, C.		
Dog	Initial Count	Lowest Count During Period of Study (6 to 7 Hr.)	Absolute Drop in Count	Initial Temp.	Highest Temp. During Period of Study	Fall in Temp.
8-D	13,150	12,600	— 550	38.55	38.50	—0.05
18-D	8,000	7,500	— 500	38.65	38.55	—0.10
12-D	6,350	6,500	+ 150	38.75	38.50	—0.25
4-27	10,050	9,150	— 900			
4-23	7,900	6,825	—1,075			
4-19	11,675	10,775	— 900			
4-27	16,500	13,200	—3,300			
7-00	8,150	7,250	— 900			
Average	10,222	9,225	— 997			—0.13

Necrosin when purified further as described previously[13] fails to reduce the number of circulating leukocytes. This is clearly seen in table 4. The introduction of varying amounts of necrosin is utterly unable to decrease the white blood cell count. If anything, there is a slight, probably insignificant, rise in the absolute number of leukocytes (table 4).

TABLE 4.—*Effect of Purified Necrosin on the White Blood Cell Count*

	White Blood Cell Counts		
Dog	Basal Count	Lowest Count Within Several Hours After Injection of Necrosin	Change in Count
6-D	10,600	10,600	0
6-D	21,350	28,250	+6,950
15-D	18,000	15,700	—2,300
28-D	5,500	8,000	+2,500
29-D	6,250	6,850	+ 600
Average	12,340	13,880	+1,540

Pyrexin, on the other hand, not only induces a marked rise in temperature, averaging 1.7 C., but causes a marked reduction of the number of circulating leukocytes in the dog (table 5). Boiling pyrexin in no way alters its pyrogenic and leukopenic properties (table 5). The doses of pyrexin employed ranged from 11 to 66 mg. The average basal leukocytic count was 12,625. The lowest level reached, usually in the first two hours after the injection of pyrexin, averaged 2,645 cells—an average drop of 9,980. A typical experiment is illustrated in chart 2. It is interesting to note that several hours after the injection of pyrexin following the leukopenic phase leukocytosis may develop, (chart 2). The

exact mechanism of this development is not clear, but it is now under investigation.[16] The evidence presented in table 5 indicates that the leukopenic factor in exudate (table 1) is probably closely associated with the pyrogenic factor, pyrexin. None of

TABLE 5.—*Effect of Pyrexin on the White Blood Cell Count*

Dog	Dose of Pyrexin, Mg.	White Blood Cell Counts			Change in Temperature, C.		
		Basal Count	Lowest Count	Drop in Count	Basal Temp.	Maximal Temp.	Rise in Temp.
8-D	35 (boiled)	11,750	3,450	8,300	38.75	40.45	+1.7
12-D	24 (boiled)	11,650	2,500	9,150	38	40.6	+2.6
18-D	45 (boiled)	11,900	2,050	9,850	38.6	39.95	+1.35
12-D	23	8,650	1,700	6,950	38.55	40.15	+1.6
12-D	29	16,650	2,600	14,050	38.75	41.2	+2.45
8-D	45	10,250	2,750	7,500	38.45	40.0	+1.55
17-D	24	15,600	1,200	14,400	38.96	40.5	+1.55
18-D	11	8,650	4,150	4,500	38.7	39.0	+0.3
18-D	66	10,000	2,150	7,850	38.7	40.55	+1.85
31-D	54	21,150	3,900	17,250	38.05	40.0	+1.95
Average.....		12,625	2,645	9,980			+1.7

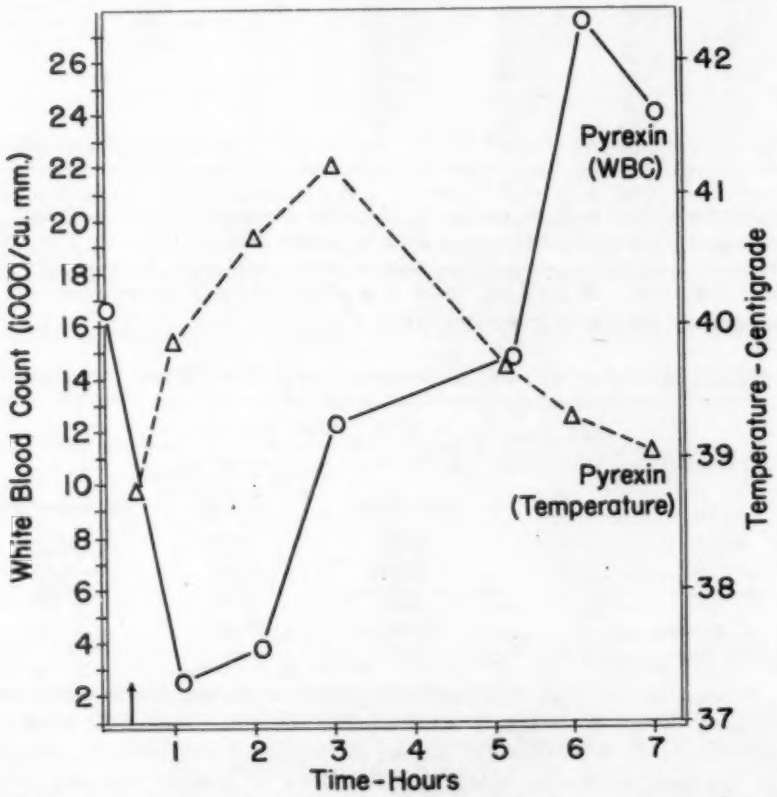

Chart 2.—Effect of pyrexin on the white blood cell count and on the temperature (dog 12-D).

16. The secondary leukocytosis following the injection of pyrexin is perhaps referable to a release into the circulation from various tissues of leukocytes trapped during the leukopenic phase (see footnote at end of this article).

the other fractions studied—for instance, the euglobulin or the pseudoglobulin—induces leukopenia in dogs. The whole exudate and pyrexin, derived from it, seem to be the materials capable of producing consistent leukopenia in dogs.

An attempt was made to determine whether the leukopenic property of pyrexin represents a chemical factor different from the pyrogenic one, or whether it pertains to the same factor as the pyrogenic property. The close association of the two effects may well indicate that one is dealing with a single substance. The fact that pyrexin can be boiled without diminishing its activity[15] and unpublished studies on the amino nitrogen before and after hydrolysis have indicated that one is possibly dealing with a polypeptide, but one which possibly is linked with some prosthetic groupings.[17] It seemed reasonable to me that one may be concerned with a mixture of polypeptides in the partially purified pyrogenic and leukopenic material termed pyrexin. For this reason pyrexin obtained from exudative material as described in an earlier communication[15] was partially hydrolyzed with tenth-normal hydrochloric acid for ten to fifteen minutes. It was observed that a dissociation occurred. By this procedure the pyrogenic property of pyrexin can be essentially eliminated, while the leukopenic activity is left practically intact. The actual scheme of extraction utilized is conveniently arranged in a diagrammatic form:

SCHEME OF THE EXTRACTION OF THE LEUKOPENIC FACTOR

Exudate
|
$(NH_4)_2SO_4$ at one-third saturation
|
Precipitate
|
Treated with distilled water
|
Shake
|
Insoluble material
|
Dialyze until free of $SO_4^=$
|
Pyrexin (dried by freezing)
|
Reflux with 0.1 NHCl for 10 to 15 minutes
|
Cool
|
With N NaOH adjust to p_H 10 or 10.3
|
Concentrate on steam bath to about $\frac{1}{10}$ volume
|
Dialyze
|
Evaporate residue to dryness on steam bath or dry freeze
|
Leukopenic fraction

After the refluxing of 20 to 25 mg. of pyrexin in 100 cc. of tenth normal hydrochloric acid, the material is cooled and brought to an alkaline p_H with normal sodium hydroxide. After the material has been concentrated, it is dialyzed against tap water to dispose of the excess of hydrochloric acid and sodium hydroxide.

17. A prosthetic group is a non-nitrogenous group linked to either a protein or a protein-derived molecule.

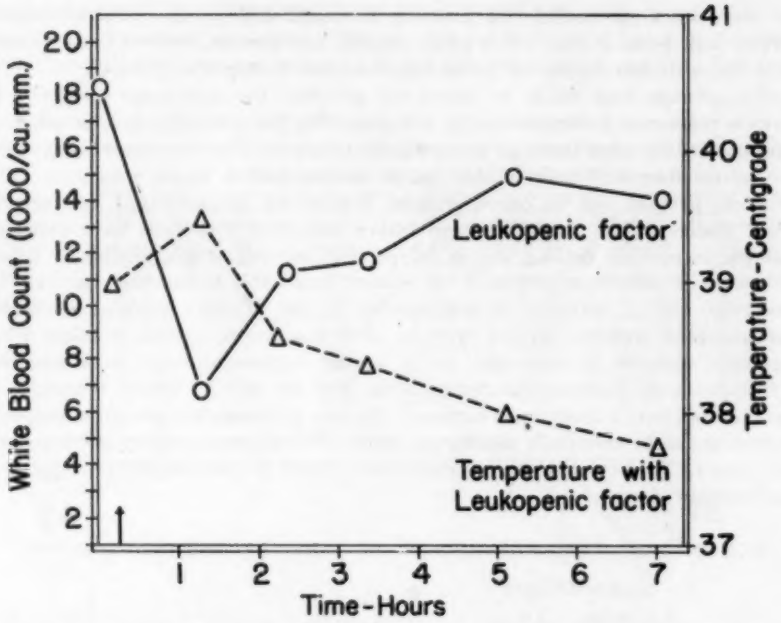

Chart 3.—Dissociation of the leukopenic factor from the pyrogenic factor in pyrexin. The essential dissociation was accomplished by incomplete hydrolysis of pyrexin (dog 26-D). Although the effect of the leukopenic factor is evident, the pyrogenic factor has to a large extent been inactivated.

TABLE 6.—*Dissociation of the Leukopenic Factor from the Pyrogenic Factor in Pyrexin*

	White Blood Cell Counts			Change in Temperature, C.		
Dog	Basal Count	Lowest Count Within 1 to 6 Hr. After Injection of Material	Drop in Count	Basal Temp.	Maximal Temp.	Change in Temp.
8-D	12,300	7,250	5,050	39.0	38.8	—0.2
18-D	10,250	5,150	5,100	38.45	38.95	+0.5
18-D	10,350	3,900	6,450	38.95	39.55	+0.6
18-D	10,450	4,400	6,050	38.35	38.75	+0.4
8-D	12,400	4,100	8,300	38.75	39.3	+0.55
18-D	12,600	7,350	5,250	38.85	38.9	+0.06
18-D	15,300	7,800	7,500	38.75	38.85	+0.1
18-D*	8,500	4,250	4,250	38.55	39.05	+0.5
31-D*(9 mg.)	22,000	13,600	8,400	38.35	38.5	+0.15
8-D*	10,800	2,950	7,850	38.8	38.8	0
8-D*(2 mg.)	11,250	5,400	5,850	38.8	38.5	—0.3
26-D*	17,800	6,800	11,000	38.95	39.45	+0.5
18-D†	9,200	4,400	4,800	38.55	39	+0.45
18-D‡	6,400	4,300	2,100	38.35	38.55	+0.2
18-D¶	5,450	4,300	1,150	38.8	38.25	—0.55
18-D¶	9,900	9,900	0	38.8	38.25	—0.55

* The leukopenic factor was obtained after dialyzing to remove the inorganic materials used in its preparation. The leukopenic factor was dried and the amount of material injected is indicated.

† The period of hydrolysis in tenth-normal hydrochloric acid was one hour instead of the usual ten to fifteen minutes.

‡ Hydrolysis was carried on for one hour in twice normal hydrochloric acid.

¶ The leukopenic factor was dissociated from the pyrogenic factor by hydrolyzing the latter with 50 per cent hydrochloric acid in an autoclave.

This fraction is now highly leukopenic and is essentially nonpyrogenic. The data concerning the dissociation of the original pyrexin are assembled in table 6. In the first seven experiments the leukopenic factor was obtained without dialyzing to separate the excess inorganic material. Nevertheless the leukopenic factor was as effective as when the excess inorganic material was disposed of by dialysis. This is readily seen by comparing the first seven experiments with the next five experiments on table 6 (marked with a single asterisk). In brief, while the leukopenic activity was maintained, the pyrogenic capacity had become negligible; the two factors had been dissociated from each other. The leukopenic fraction can either be dried on a steam bath or lyophilized in a dry

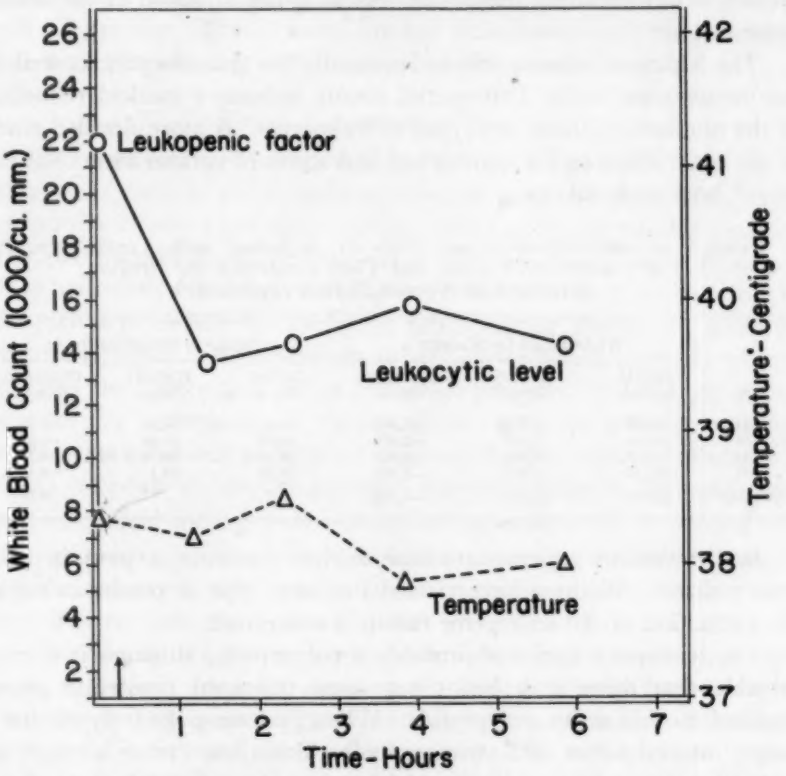

Chart 4.—Dissociation of the leukopenic factor from the pyrogenic factor of pyrexin. The latter factor has been completely inactivated, while the leukopenic factor has been left essentially intact (dog 31-D).

freezing apparatus. When obtained by such a technic, the leukopenic factor of exudate is found to induce leukopenia without being pyrogenic (table 6). The dissociation of pyrexin to yield a potent leukopenic factor while inactivating the pyrogenic property is illustrated in charts 3 and 4.

Control observations have been made by refluxing with tenth-normal hydrochloride acid alone and then rendering the product alkaline with normal sodium hydroxide. These preparations failed to manifest either leukopenic or pyrogenic activity in dogs (table 7).

SOME PROPERTIES OF THE LEUKOPENIC FACTOR

The leukopenic factor of exudate apparently reduces the number of white blood cells throughout the circulating blood stream, and therefore the effect does not seem to be referable to a redistribution of white cells in the circulation. This was shown to be the case by injecting the leukopenic factor into the blood stream and then taking samples of blood both from a peripheral vessel, that of the ear, and from the heart. Both the peripheral and the systemic blood sample revealed similarly a marked reduction in circulating leukocytes following the injection of the leukopenic factor.[18]

The leukopenic factor affects apparently the granulocytes as well as the mononuclear cells. Differential counts indicate a marked reduction in the numbers of these two types of leukocytes. A more detailed study of the exact effect on the numbers of leukocytes of various forms has not as yet been undertaken.

TABLE 7.—*Control Observations Made by Refluxing with Tenth-Normal Hydrochloric Acid Alone and Then Rendering the Product Alkaline with Normal Sodium Hydroxide*

	White Blood Cell Counts			Change in Temperature, C.		
Dog	Initial Count	Lowest Count	Change in Count	Initial Temp.	Highest Temp.	Change in Temp.
8-D	11,800	13,700	+1,900	38.2	38.55	+0.35
12-D	16,750	13,950	—2,800	38.85	39.35	+0.5
31-D	13,350	10,700	—2,650	38.45	38.75 (2 hr.)	+0.3
18-D	12,150	10,950	—1,200	38.50	38.1	—0.4
Average	13,513	12,325	—1,188			+0.19

In the various experiments four distinct fractions of pyrexin have been utilized. All these have yielded the same type of results as far as the extraction of the leukopenic factor is concerned.

The leukopenic factor is probably a polypeptide, although it is conceivable that there may be as yet some unknown prostethic group attached to this active polypeptide. When pyrexin is hydrolyzed for a longer interval either with stronger hydrochloric acid (twice normal) or with 50 per cent hydrochloric acid in an autoclave, both the leukopenic and the pyrogenic property are inactivated. This is indicated in table 6 (experiments marked with the symbols ‡ and ¶). These experiments indicate that brief hydrolysis with relatively weak hydrochloric acid inactivates only the pyrogenic factor (pyrexin), whereas prolonged hydrolysis with stronger hydrochloric acid inactivates also the leukopenic

18. The effect of the leukopenic factor is evidently not referable to hemodilution. For instance, the number of red corpuscles was hardly altered when 10 mg. of pyrexin was injected into the circulating blood of a dog. Whereas the white cell count dropped from 13,950 to 2,800 in forty-four minutes the red cell count remained essentially constant during a period of more than three hours.

factor (table 6). This suggests that possibly the latter has more peptide linkages than pyrexin. Further chemical studies are necessary, however, and will accordingly be undertaken.

Finally, preliminary determinations of nitrogen and measurements of amino nitrogen before and after hydrolysis, performed by Dr. Frederick Bernheim, indicate that one is quite likely dealing with a polypeptide. Nitrogen determined on the leukopenic factor was found to be 16 per cent. The amino nitrogen before hydrolysis was found in three analysis to average 0.85 per cent, whereas after hydrolysis the average was 5.2 per cent. These results are not inconsistent with the possibility that the leukopenic factor of exudate is a polypeptide.

COMMENT

The observations indicate that the pyrogenic substance, pyrexin, in its present state of purification contains at least two properties, which seem to be closely associated with each other in that particular material. The two properties can be dissociated by subjecting pyrexin to incomplete hydrolysis for a short interval in relatively weak hydrochloric acid. By such a procedure the pyrogenic factor can be inactivated while the leukopenic factor is left essentially intact. Nevertheless, since this process involves the obliteration of the pyrogenic property, it is not yet known whether the leukopenic and the pyrogenic capacities are two properties of the same substance or whether each represents a separate substance in the as yet relatively impure pyrexin. Further studies on the purification of pyrexin and the eventual recovery of separate material entities with their respective pyrogenic and leukopenic properties can definitely establish the concept of two separate factors in the same substance or that of two different substances. Such studies are now in progress.

The finding of a leukopenic factor in inflammatory exudates, particularly at an acid p_H, is of significance. It may help in further understanding of the leukopenic disturbance in various clinical disorders, such as typhoid, tuberculosis and influenza, as well as in many other pathologic conditions. It is conceivable that the constant production of this leukopenic factor at the site of severe cellular injury may yield a continuous state of leukopenia in the circulation. It is perfectly possible that the ultimate cytologic picture of either leukopenia or leukocytosis in the blood stream with inflammation may depend on whether the release of the leukocytosis-promoting factor or that of the leukopenic factor predominates at the site of injury.

Finally, it has been pointed out in an earlier study [19] that the fundamental stereopattern of inflammation is largely referable to various

19. Footnotes 8 and 13.

common denominators liberated from cells previously injured by an irritant. When these basic substances have reached a sufficient concentration in the injured cells and have been liberated, their effect becomes evident. They include leukotaxine the leukocytosis-promoting factor, necrosin, pyrexin, dextrose [20] and, now may finally be added, the leukopenic factor. It is the liberation of these common denominators which determines the final picture of inflammation. In brief, this concept refers the problem of inflammation to a biochemical study of the injured cell.

CONCLUSION

A leukopenic factor is present in inflammatory exudates of dogs, particularly if the exudate gives an acid reaction. When injected into the blood stream of a normal animal, the leukopenic factor causes a rapid fall in the number of circulating leukocytes. In its present state of purification it is found as a rule to be closely associated with pyrexin, the substance primarily concerned in the development of the fever associated with inflammation. It can be dissociated from the pyrogenic factor by subjecting pyrexin to incomplete hydrolysis, which inactivates the pyrogenic factor. The evidence on hand does not as yet prove conclusively that the leukopenic factor is a substance separate from pyrexin. It may be only a separate factor in pyrexin. Further studies are in progress to establish this point. Preliminary observations indicate the probable polypeptide nature of the leukopenic factor.[21]

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20. Am. J. Physiol. **134**:517, 1941; **138**:396, 1943.

21. In subsequent studies, made since this communication was sent to the ARCHIVES for publication, it has been found that, although the leukopenic factor of inflammatory exudate is mostly found in close association with pyrexin, yet it can at times be recovered to some extent in other fractions of exudative material, indicating that it is apparently not exclusively found in association with pyrexin. Furthermore, additional studies seem to indicate that the leukopenic factor of exudates does not primarily deplete the bone marrow, but rather the mechanism involved appears to be a rapid trapping of leukocytes in the alveolar walls of the lungs, in the sinusoids of the liver and apparently in the spleen. The latter fact may be of significance in the further understanding of the mechanism involved in the acute splenic tumor accompanying numerous inflammatory processes.

Case Reports

OBSTRUCTION OF THE AORTIC ISTHMUS BY A CALCIFIED THROMBUS

HERBERT D. AXILROD, M.D.
NEW HAVEN, CONN.

OBSTRUCTION of the thoracic portion of the aorta is uncommon, and in the majority of instances it is due to coarctation.[1] Fresh thrombi in this location have been described, but such a thrombus does not occlude the vessel to any pronounced degree.[2] The only example of significant narrowing of the thoracic aorta by an organizing thrombus was reported by Aubertin.[3] An instance of almost complete stenosis of the aortic isthmus by a calcified thrombus is presented in the following communication.

REPORT OF A CASE

A 40 year old Negro sandblaster was found to have a positive serologic test for syphilis at an army induction center in February 1943. He received thirty-three injections of a bismuth preparation and six injections of oxophenarsine hydrochloride at irregular intervals during the following ten months.

Initial symptoms in August 1943 were mild substernal discomfort and numbness and tingling in the arms on exertion. Two months later there were additional symptoms of suffocation, palpitation and weakness on exertion. At this time the systolic blood pressure was 200 mm. of mercury. In January 1944, after a particularly severe attack of the previously noted symptoms, the patient came to the New Haven Dispensary. At no time did he suffer from dyspnea, orthopnea, edema, headaches or epistaxis. He had continued to perform heavy manual labor.

He was an extremely well developed, well nourished, middle-aged Negro, who appeared in no acute distress. The fundi showed sharply outlined disks and slightly narrowed, tortuous vessels. The lungs were clear to percussion and auscultation. The forceful point of the maximal impulse of the heart was localized in the fifth interspace in the anterior axillary line. No thrills were felt. The sounds were of good quality, with the aortic greater than the pulmonic second sound. The rate was 66 per minute; the rhythm, regular. There was a harsh systolic apical murmur, transmitted to the axilla, as well as a loud harsh systolic murmur in the aortic region, transmitted to the cervical vessels on the right side. The blood pressure was 185 systolic and 70 mm. diastolic. The liver was palpable

From the Department of Pathology, Yale University School of Medicine.

1. Hamilton, W. F., and Abbott, M. E. Am. Heart J. **3**:381 and 574, 1928. Lewis, T.: Heart **14**:205, 1933.

2. Welch, W.: Embolism and Thrombosis, in Allbutt, T. C., and Rolleston, H. D.: System of Medicine, New York, The Macmillan Company, 1909, vol. 15. Desclin, L.: Frankfurt. Ztschr. f. Path. **40**:520, 1930. Hancock, J. C.: J. Iowa M. Soc. **31**:543, 1941.

3. Aubertin, M. C.: Ann. de méd. **10**:454, 1921.

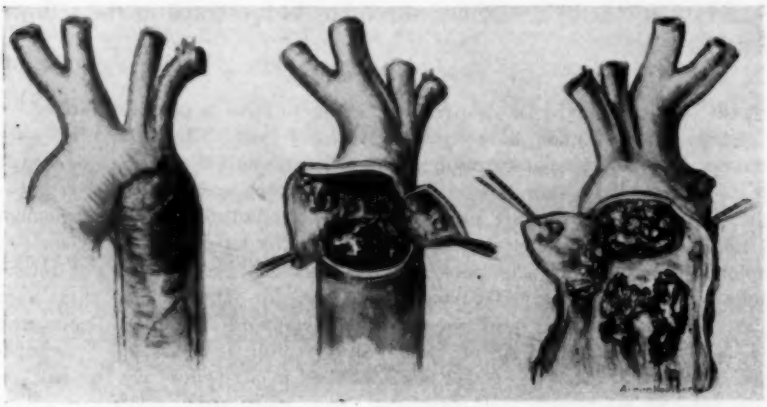
2 fingerbreadths below the costal margin. No edema of the extremities was noted, and the pulsations in the dorsalis pedis artery were of good quality. Otherwise the examinations, including that of the urine, showed no marked deviations from the normal.

The clinical diagnosis was cardiovascular syphilis with syphilitic aortitis, coronary insufficiency, dilatation of the aorta, left-sided cardiac hypertrophy and hypertension.

The patient was subsequently followed in the dispensary, where a roentgen examination revealed cardiac enlargement and calcification of the aortic knob. Treatment with glyceryl trinitrate afforded great relief. Arteriosclerotic heart disease and calcific aortic stenosis were added to the diagnosis.

On Nov. 16, 1944, the patient reached the emergency room of the New Haven Hospital coughing up frothy, blood-tinged sputum. Treatment for acute pulmonary edema with theophylline ethylenediamine, morphine and oxygen did not prevent a prompt fatal outcome.

Pertinent Anatomic Findings.—The body was muscular and well developed. There was bilateral hydrothorax. The heart weighed 600 Gm., and the left



Drawings of the aorta with obstruction at the isthmus.

ventricular muscle measured 17 mm. in thickness. The free edges of the cusps of the aortic valve were slightly thickened, and the commissures were only 2 mm. wide. The aortic valve measured 7.5 cm. in circumference and did not appear insufficient. The ostium of the left coronary artery was narrowed to 2 mm. in diameter.

The aorta was occluded for a distance of 3 cm. beyond the mouth of the subclavian artery by a stony-hard, irregularly shaped mass which, as seen on cross section, left a crescent-shaped lumen measuring 3 mm. at the widest point (figure). The decalcified microscopic preparation showed the occluding material to consist of densely packed fibrin with organization at the periphery and small zones of bone formation. The elastic media at this level was interrupted by vascular scars. The adventitia consisted of a thick layer of acellular, dense fibrous connective tissue, and there were accumulations of plasma cells and lymphocytes around the vasa vasorum. Similar syphilitic lesions, as well as intimal fibrosis and atheromatosis, were present in other portions of the thoracic aorta. Smaller mural calcified thrombi occurred above and below the obstruction. The internal mammary, intercostal and long thoracic arteries were not enlarged.

COMMENT

The nature of the aortic lesion indicated that obstruction had been present for a considerable period. It is not uncommon for extreme stenosis of the aortic valve to exist for a long time with only slight circulatory embarrassment. In such circumstances there is no opportunity for the development of collateral circulation. These observations may explain the fact that the narrow lumen of the aorta in this patient allowed adequate circulation until failure of the left ventricle occurred.

SUMMARY

A calcified thrombus was observed in a patient with cardiovascular syphilis which produced almost complete obstruction of the isthmus of the aorta.

General Reviews

TROPICAL DISEASES

Involvement of the Nervous System

W. S. CHALGREN, M.D., and A. B. BAKER, M.D.
MINNEAPOLIS

WITHIN recent years, owing to the shifting of large numbers of the population to many points throughout the world, particularly to the subtropics and the tropics, a great deal of interest has been manifested in the various diseases most characteristic of these regions. As a result, many reports and publications have appeared dealing with the subject of the tropical diseases. Curiously enough, most of the recent literature, even when exhaustive, has ignored almost completely the possible and often probable involvement of the nervous system. It has been recognized that certain of these diseases, such as the rickettsial infections and malaria, produce definite encephalitis, but the fact is overlooked that almost every tropical disease may involve the nervous system to produce encephalic changes with possible residual damage of the brain. Such complications can well be of utmost importance in the final evaluation of the recovery of the patients, especially when large proportions of the population are being exposed and infected. It is for the purpose of emphasizing the encephalic complications of the tropical diseases that the present review was undertaken. Since many excellent reports have already been published describing the general characteristics of these diseases, no attempt will be made in the present review to repeat the data contained in those reports. Only such features will be included as appear necessary to introduce the encephalic complications. In attempting such a study it was felt to be most convenient to group the various diseases according to the natures of the causative organisms.

FILTRABLE VIRUSES

Yellow Fever.—Yellow fever is an acute infectious type of jaundice caused by a virus which is transmitted from man to man by the mosquito Aedes aegypti. It occurs in endemic form as jungle yellow fever in vast areas of tropical South America and Africa, and in epidemic form in various urban communities in equatorial Africa and tropical South and Central America. The onset of the disease is usually sudden, with slight

From the Department of Neuropsychiatry, The Medical School, University of Minnesota.

chills and fever, headache, pains of the muscles, nervousness and anxiety. These symptoms are followed in a day or two by albuminuria, oliguria, increasing fever, nausea, vomiting and severe prostration. Jaundice appears on the fourth or the fifth day, accompanied by bleeding from the gums and hemorrhages of the stomach and the intestine. Death usually occurs from the sixth to the ninth day, preceded by delirium, prostration and coma. The mortality rates in epidemics have been reported as from 5 to 10 per cent up to 80 per cent.

The essential lesions of yellow fever are found in the liver and are characterized by midzonal necrosis, fatty degeneration and the presence of "councilman bodies." Less striking changes are noted in the spleen, the kidneys, the heart, the lungs and the gastrointestinal tract. Although the virus of yellow fever is highly neurotropic, there have been but few reports dealing with the pathologic involvement of the nervous system. Most investigators have examined the brain superficially and have stated that there were no significant changes (Noguchi [1]; Mackenzie [2]). Schmidt [3] in 1879 expressed the opinion that some patients with yellow fever died from "congestion of the brain." He found fatty degeneration and fatty infiltration of the cortical neurons as well as vascular congestion. Similar observations were reported by Pothier [4] in 1905-1906.

Jacob, Fialho and Villela [5] in the first careful study of the brain in yellow fever found definite encephalitis in 14 cases. They observed mild meningeal infiltration with lymphocytes and macrophages and marked fatty degeneration of the ganglion cells in the cortex, the basal nuclei, the hippocampus and the dentate nucleus. Similar fatty degeneration was found in the endothelial cells of the walls of blood vessels. Many of the neurons were swollen and showed chromatolysis, while others were pyknotic and fragmented. Demyelination and gliosis were noted about many of the blood vessels. In 4 cases perivascular granulomas were found within the gray matter of the medulla and the pons; they were described as focal collections of glial cells intermixed with lymphocytes and surrounded by diffuse glial proliferation. In 6 cases there were marked degeneration and focal changes in the cerebellar cortex. These changes varied from moderate disappearance of Purkinje cells to severe diffuse gliosis with perivascular demyelination.

Stevenson [6] studied the brains of 20 persons who died of yellow fever. He found perivascular hemorrhages in all, most numerous in the

1. Noguchi, H.: J. Exper. Med. **29**:547, 1919.
2. Mackenzie, I.: J. Trop. Med. **30**:218, 1927.
3. Schmidt, H. D.: M. Rec. **16**:44, 1879.
4. Pothier, O. L.: New Orleans M. & S. J. **58**:394, 1905-1906.
5. Jacob, A.; Fialho, A., and Villela, E. L.: Deutsche Ztschr. f. Nervenh. **111**:111, 1929.
6. Stevenson, L. S.: Arch. Path. **27**:249, 1939.

subthalamic and periventricular regions at the level of the mamillary bodies. Slight perivascular edema was noted in some cases and perivascular lymphocytes in others. In only 1 case did he note any loss of nerve cells. Reactive changes in the microglia and the astroglia were slight.

Nicolau, Mathis and Baffet [7] in a case report described marked glial proliferation forming perivascular "cuffing" within the brain substance, with accompanying mononuclear infiltration. Both nerve cells and glial elements contained characteristic oxyphilic inclusion bodies of a type described by them in a previous paper.[8]

In 1928 Stokes, Bauer and Hudson [9] discovered a susceptible laboratory animal, and in 1930 Theiler [10] found that the virus of yellow fever is infectious for the brain of the mouse. These discoveries marked a new era in research on yellow fever. Numerous pathologic studies have been made of encephalitis in monkeys and mice experimentally infected with the virus of yellow fever. Aside from the original study by Hudson and his co-workers,[9] in which no abnormal changes were observed, well marked encephalitic changes have been described. Goodpasture,[11] using monkeys, found extensive acute degeneration and necrosis of ganglion cells. Some of the nuclei of these cells contained coarse acidophilic inclusions. Polymorphonuclear leukocytes diffusely infiltrated the cortical tissues, and there was a thin perivascular mononuclear infiltration. Petechiae were numerous. Lloyd and Penna [12] noted degenerative changes in the nerve cells of the cortex, brain stem and spinal cord, with acidophilic particles in the cell nuclei. There was definite perivascular lymphocytic infiltration of the brain and the spinal cord. Accompanying these changes was a considerable amount of interstitial glial cell infiltration. Findlay and Stern [13] observed the following changes: generalized microglial proliferation, nerve cell changes, which in the monkey consisted of degeneration and neuronophagia, and the presence of a perivascular inflammatory exudate consisting of lymphocytes.

It is apparent from these reports that well defined encephalitis occurs in both man and experimental animal in yellow fever. Particularly in the experimental animal does this virus show highly neurotropic properties.

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7. Nicolau, S.; Mathis, M., and Baffet, O.: Bull. Soc. path. exot. **30**:615, 1937.
8. Nicolau, S.; Kopciowska, L., and Mathis, M.: Ann. Inst. Pasteur **53**: 455, 1934.
9. Stokes, A.; Bauer, J. H., and Hudson, N. P.: Am. J. Trop. Med. **8**:103, 1928.
10. Theiler, M.: Ann. Trop. Med. **24**:249, 1930.
11. Goodpasture, E. W.: Am. J. Path. **8**:137, 1932.
12. Lloyd, W., and Penna, H. A.: Am. J. Trop. Med. **13**:1, 1933.
13. Findlay, G. M., and Stern, R. O.: J. Path. & Bact. **40**:311, 1935.

Dengue.—Dengue is a common virus disease occurring in both epidemic and endemic form in subtropical and tropical localities. Under favorable climatic conditions, such as warm, wet weather, epidemics of the infection may reach as far north as Philadelphia. The disease was first described in Cairo, Egypt, and Batavia, Java, in 1779 (Beylon [14]) and in Philadelphia in 1780 (Griffetts and Griffetts [15]). There have been nine epidemics in the United States, the last occurring in the Southern states in 1922-1923, at which time 29,827 cases were reported in Louisiana (Scott [16]) and 82,681 cases in Florida (Richardson [17]). The virus is spread chiefly by the mosquito Aedes aegypti, although Aedes albopictus (the most common oriental mosquito) can also transmit it.

The clinical picture in dengue can be kaleidoscopic, producing disturbances of the gastrointestinal tract, the heart, the adrenal glands and the nervous system, any one of which may dominate the picture (King [18]). The two most characteristic features, however, are the paroxysms of biphasic temperature and the severe pain occurring in the muscles and the joints. Because of these features the disease is occasionally referred to as "breakbone fever," "saddle back fever" and "three day fever." The onset is generally acute, resulting in a high temperature, chills, severe prostration and occasionally a cutaneous rash. In spite of the severe morbidity, most patients recover, and therefore pathologic studies of this illness are few. However, even clinically it is apparent that the nervous system is frequently and often severely implicated. As a matter of fact, some investigators, particularly Apostolopoulos [19] and Pamboukis,[20] expressed the belief that the virus of dengue is chiefly neurotropic and that the clinical manifestations result indirectly from the action of the virus on the nervous system. Aside from the headaches, complaints referable to all parts of the nervous system have been described, such as photophobia, lethargy, paresis, convulsions and even psychic disturbances (Gill [21]; Ghiannoulatos [22]; Richardson [17]; Faver [23]; Bargy [24]).

Studies on the nervous system are not too common, because of the low mortality rate. Catsaras [25] reported cerebral changes in 3 cases of

14. Beylon, D., in Strong,[135] vol. 2.
15. Griffetts, T. H. D., and Griffetts, J. J.: Pub. Health Rep. **46**:2725, 1931.
16. Scott, L. C.: J. A. M. A. **80**:387, 1923.
17. Richardson, S.: Tr. Am. Ophth. Soc. **31**:450, 1933.
18. King, W. W.: New Orleans M. & S. J. **69**:564, 1916-1917.
19. Apostolopoulos, K. G.: München. med. Wchnschr. **77**:265, 1930.
20. Pamboukis, G.: Schweiz. Arch. f. Neurol. u. Psychiat. **26**:51, 1930.
21. Gill, W. D.: Arch. Ophth. **57**:628, 1928.
22. Ghiannoulatos, G. P.: Rev. neurol. **38**:599, 1931.
23. Faver, M.: J. Florida M. A. **24**:395, 1938.
24. Bargy, M.: Bull. et mém. Soc. franç. d'opht. **42**:293, 1929.
25. Catsaras, J.: Arch. f. Schiffs- u. Tropen-Hyg. **35**:278, 1931.

dengue. Histologically, the cortical neurons were swollen and irregular in contour. They often consisted of irregular masses without nuclei. No inflammatory changes were observed. Melissinos [26] reported 3 cases in which the patients presented convulsions, lethargy and coma. The histologic changes were consistent in all cases and were divided by the author into three types, namely: degenerative, inflammatory and hemorrhagic. The degenerative changes consisted of neuronal alterations, evidenced by swelling and chromatolysis. Hemorrhagic foci were observed scattered throughout the brain. In many of these foci the vessels showed early inflammatory changes with swelling of the vascular endothelium and beginning extravasation of red cells. Inflammatory changes were present independently of the hemorrhagic foci and consisted of vascular hyperemia, perivascular leukocytic infiltration, glial reactions and endothelial proliferation. Numerous small cellular collections were scattered throughout the tissues. These were composed of both leukocytes and glial elements. No areas of softening or demyelination were observed.

RICKETTSIAL DISEASES

The rickettsias are minute bacteria-like micro-organisms which live and multiply intracellularly in the tissues of arthropod or animal hosts. The rickettsias which cause diseases of man may be divided into four groups on the basis of epidemiologic, pathologic and immunologic studies. They are: the typhus group, which are transmitted by body lice and fleas; the Rocky Mountain spotted fever group, transmitted by ticks; the tsutsugamushi fever group, transmitted by mites, and the Q fever group, which may be transmitted either by ticks or by droplets from infected persons.

With the exception of Q fever, the rickettsial diseases are clinically, pathologically and immunologically quite similar. They are characterized by sudden onset, rash, fever of fairly well defined duration, various nervous and mental disturbances and prostration. The rickettsias appear to have definite neurotropic tendencies, invading the brain to produce a primary encephalitic process. The principal pathologic changes are proliferative and inflammatory changes in the smaller branches of the vascular system.

Epidemic and Endemic Typhus.—Epidemic typhus is an acute infectious disease caused by Rickettsia prowazeki and transmitted from man to man by the louse (Pediculus humanus). It is worldwide in distribution, and severe epidemics are associated with war, overcrowding and poor hygienic conditions. Endemic foci of this disease are present in the highlands of Central and South America, North Africa and parts of

26. Melissinos, J.: Arch. f. Schiffs- u. Tropen-Hyg. **41**:321, 1937.

Central and South Africa, in southern and eastern Europe and in Asia Minor, India and China.

There is no question that the nervous system manifestations of typhus fever form an important part of the symptom complex (Gerhard [27]; Murchison [28]; Hampeln [29]; Curschmann [30]; Devaux [31]; Rabinovitch [32]; Baeyer [33]; Skliar [34]; Munk [35]; Stockert [36]). Devaux in commenting on the epidemic in Rumania in 1919 stated, "There are few patients who do not present either during the febrile period or during convalescence some more or less grave and persistent trouble indicating central nervous system and peripheral nerve involvement." He described the neurologic complications as consisting during the first week of delirium, convulsions, monoplegias and hemiplegias. During the second week the symptoms showed bulbar localization of the infection. Most neurologic symptoms occurred during convalescence. He reported 215 cases.

Pathologic changes in the central nervous system in typhus were first described by Popoff,[37] in 1875, and by Ivanovskii,[38] in 1876. They both described widespread nodular collections of wandering cells in the pericellular and perivascular spaces of the cerebral cortex, accompanied by an interstitial inflammatory reaction. They observed an increase of nodular elements in the region of the ganglion cells with atrophy and degeneration of the latter.

It was not until 1914 that further studies of the central nervous system in typhus were reported. In that year Alphejewski [39] reported a case in which he found round cell infiltration of the meninges and brain tissue as well as proliferation of cells within the walls of blood vessels, producing obliteration of the lumens, and formation of granulomas resembling miliary gummas. Many of the nerve cells showed swelling, chromatolysis and neuronophagia. Scattered areas of demyelination were visible throughout the cerebral cortex. Prowazek [40] in 1915 studied in particular the structure of the cerebral nodules in this disease and believed that

27. Gerhard, W. W.: Am. J. M. Sc. **20**:289, 1837.
28. Murchison, C.: A Treatise on the Continued Fevers of Great Britain, London, Longmans, Green & Company, 1873.
29. Hampeln, P.: Deutsches Arch. f. klin. Med. **26**:238, 1880.
30. Curschmann, H., in Nothnagel, H.: Encyclopedia of Practical Medicine, Philadelphia, W. B. Saunders and Company, 1901, vol. 1, p. 475.
31. Devaux, A.: Lancet **1**:567, 1919.
32. Rabinovitch, J. S.: Ztschr. f. d. ges. Neurol. u. Psychiat. **115**:34, 1928.
33. von Baeyer, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **175**:225, 1942.
34. Skliar, N.: Monatschr. f. Psychiat. u. Neurol. **52**:21, 1922.
35. Munk, F.: Med. Klin. **36**:452, 1940.
36. Stockert, F. G.: Deutsche med. Wchnschr. **69**:506, 1944.
37. Popoff, L.: Zentralbl. f. d. med. Wissensch. **13**:596, 1875.
38. Ivanovskii, P.: J. dlya normal. i patol. gistologii **10**:93, 1876.
39. Alphejewski, N.: Sovrem. psikiat. **8**:279, 1914.
40. von Prowazek, S.: Beitr. z. klin. Infektionskr. **4**:5, 1915-1916.

they were composed of polymorphonuclear leukocytes, isolated endothelial cells, plasma cells and rarely glial elements. Benda,[41] on the other hand, held that these nodules were composed chiefly of glial cells. He noted that the individual nodule was not particularly localized to a vessel and the immediately adjacent tissue; the nodules were present throughout the brain. Ceelen [42] described the formation of the nodule as beginning with proliferation and swelling of the vascular endothelium, associated with proliferation of the adventitial cells and perivascular collection of lymphocytes. He emphasized in particular that the neuroglia participate in the formation of the nodule, that the nodule is constantly associated with a vessel and that there are severe ganglion cell and myelin changes in the vicinity of nodules.

Following these reports, a great number of articles appeared describing the changes in the nervous system in typhus. Many of these are summarized in the works of Wolbach, Todd and Palfrey,[43] Ceelen,[44] Dawydowskie [45] and Hirschberg.[46]

Wolbach, Todd and Palfrey [43] examined 39 cases of epidemic typhus in Poland in 1919-1920 and gave an excellent description of the pathologic alterations of the nervous system. Macroscopically most of the brains appeared normal, but a few showed injection of the meningeal and cortical vessels and petechiae in the basal ganglion, the pons and the midbrain.

The microscopic process was characterized by presence throughout the central nervous system of tubercle-like nodules in some way associated with small vessels. These nodules were most frequent in the medulla, the pons, the midbrain, the basal ganglions and the cerebral cortex, especially the parietal region. The nodules consisted chiefly of neuroglia cells with some polymorphonuclear leukocytes, plasma cells, occasional red cells and rarely endothelial cells. The authors felt that macrophages were important constituents of the nodules.

According to these authors, the earliest lesions of typhus are found in the capillary and precapillary endothelium, which becomes swollen and proliferated, often obliterating the lumen of the vessel. Erythrocytes frequently escape into the perivascular spaces, producing tiny hemorrhages about the vessels. Following this, a mononuclear infiltration

41. Benda, C.: Ztschr. f. ärztl. Fortbild. **12**:464, 1915.

42. Ceelen, W.: Ergebn. d. allg. Path. u. path. Anat. **19**:307, 1919.

43. Wolbach, S. B.; Todd, J. L., and Palfrey, F. W.: The Etiology and Pathology of Typhus, Cambridge, Mass., Harvard University Press, 1922.

44. Ceelen, W.: Klin. Wchnschr. **53**:530, 1916.

45. Dawydowskie, J. W.: Ergebn. d. allg. Path. u. path. Anat. (pt. 2) **20**:571, 1923-1924.

46. Hirschberg, N.: Fleckfieber und Nervensystem, in Abhandlungen aus der Neurologie, Psychiatrie, Psychologie und ihren Grenzgebieten, 1932, no. 66.

appears around the affected vessels. There is also a simultaneous reaction of the neuroglia resulting in the formation of small nodules. Polymorphonuclears are invariably present in small numbers, as are also plasma cells and a few red cells. These various lesions were interpreted as representing a definite proliferative reaction preceded by injury of and proliferation of the vascular endothelium. The authors concluded that the endothelial and the neuroglial proliferation were in direct response to the parasite of typhus carried into the nerve tissue by the migration of endothelial cells. Rickettsias were seen and described in the endothelial cells. Ganglion cell changes evidenced by chromatolysis were observed in the nuclei of the medulla and the midbrain and in the Purkinje cells of the cerebellum.

Pathologic changes similar to those reported by Wolbach, Todd and Palfrey had already been noted by a number of investigators. Fraenkel,[47] Aschoff [48] and Jarisch [49] had observed the nodules in the brain. Albrecht [50] stressed the vascular origin of the nodules and noted marked perivascular changes. Herzog,[51] Abrikossoff,[52] Jarisch [49] and Krinitzki [53] expressed the opinion that the vascular changes were due to thrombosis and that the nodules were primarily vascular in origin, secondary to a reparative process. Spielmeyer,[54] on the other hand, suggested that the nodules arose independently of any vascular pathologic process. Licen [55] also held that the glial reaction was more sensitive in typhus than the reaction of the mesodermal elements. He found bushlike glial proliferation in the molecular layers of the cerebellum, more obvious than the usual nodule formation. Dawydowskie [45] agreed with Spielmeyer that the nodules were composed chiefly of neuroglia and could be found independent of vessels. He reported observations in 70 cases. The nodules were most numerous in the medulla and were present as late as thirty days after the onset of the illness. Hassin [56] observed that most of the lesions were associated with the smaller vessels. He described the vascular changes as consisting of congestion, thrombosis, proliferation of endothelial and adventitial cells and perivascular infiltration, especially of plasma cells. The meninges were diffusely infiltrated by various hematogenous cells.

47. Fraenkel, E.: München. med. Wchnschr. **62**:805, 1915.
48. Aschoff, L.: Med. Klin. **11**:798, 1915.
49. Jarisch, A.: Deutsches Arch. f. klin. Med. **126**:270, 1918.
50. Albrecht, H.: Centralbl. f. allg. Path. u. path. Anat. **27**:247, 1916.
51. Herzog, G.: Centralbl. f. allg. Path. u. path. Anat. **29**:97, 1918.
52. Abrikossoff: Virchows Arch. f. path. Anat. **240**:281, 1922.
53. Krinitzki, cited by Dawydowskie.[45]
54. Spielmeyer, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **47**:1, 1919.
55. Licen, E.: Ztschr. f. d. ges. Neurol. u. Psychiat. **53**:199, 1919-1920.
56. Hassin, G. B.: Arch. Neurol. & Psychiat. **11**:121, 1924.

Although the characteristic nodules have been observed in all parts of the central nervous system, they occur more frequently in some areas than in others. According to a majority of authors, they are most often seen in the gray matter — the cortex and the various nuclei. They are especially numerous in the brain stem in the region of the olivary and cranial nerve nuclei. Nodules have been reported in the cord by Spielmeyer,[54] Marinesco [57] and Herzog,[51] while Dawydowskie,[48] Morgenstern,[58] Marinesco,[57] Popoff [37] and Gutmann [59] have reported vascular and proliferative changes within the peripheral nerves.

The typical lesions of typhus first make their appearance around the third and fourth days of the illness. They rapidly increase, and the maximum reaction seems to occur during the second and third weeks. According to Krinitzki,[53] the height of the nodule formation is from the tenth to the sixteenth day, following which they gradually disappear. Nicol [60] found lesions to persist as long as the eighth week, while Jarisch [49] and Dawydowskie [48] saw no lesions after the eighth week in their cases.

Endemic, or murine, typhus differs from epidemic typhus chiefly on epidemiologic grounds. It is transmitted from rat to rat and from rat to man by rat fleas. Clinically it is much the same as the epidemic form except that the symptoms are less severe and the complications are few. The pathologic studies of the central nervous system in endemic typhus have been limited to examinations of experimental animals. Mooser [61] found typical typhus nodules in the brains of guinea pigs infected with rickettsias of Mexican typhus. Similar findings were reported by Dyer and his associates.[62]

Rocky Mountain Spotted Fever.—The diseases caused by Rickettsia rickettsii, which is transmitted from the animal reservoir to man by various species of ticks, have a wide distribution and include Rocky Mountain spotted fever of North America, Tobia fever of Colombia, São Paulo exanthematic typhus of Brazil, fievre boutonneuse of the Mediterranean countries, Kenya typhus in Africa and probably South African tick bite fever and tick typhus of India. The clinical features of Rocky Mountain spotted fever are similar to those of typhus, the differences being in the nature of the rash and the duration of the fever. Disturbances of the central nervous system are generally regarded as being severe, although there have not been reported the variety of symp-

57. Marinesco, G.: Ann. Inst. Pasteur **36**:209, 1922.

58. Morgenstern: Virchows Arch. f. path. Anat. **238**:227, 1922.

59. Gutmann, A.: Deutsche med. Wchnschr. **42**:1538, 1916.

60. Nicol, K.: Beitr. z. path. Anat. u. z. allg. Path. **65**:120, 1919.

61. Mooser, H.: J. Infect. Dis. **43**:241, 1928.

62. Dyer, R. E.; Ceder, E. T.; Lillie, R. D.; Rumreich, A., and Badger, L. F.: Pub. Health Rep. **46**:2481, 1931.

toms found with typhus (Rumreich, Dyer and Badger [63]; Toomey [64]; Baker [65]; Smith and Reinhard [66]; Bennett [67]; Palatucci and Maragoni [68]).

The pathologic aspects of Rocky Mountain spotted fever have been described in detail by Lillie.[69] The fundamental lesion is vascular and is characterized by endothelial swelling and proliferation, sometimes going on to necrosis and thrombosis. There is often independent perivascular cellular infiltration. Rickettsias can frequently be seen in the swollen endothelial cells. The organs most involved are the skin, the heart, the kidney and the brain. In the brain pathologic changes are not prominent until after the tenth day of the disease. This accounts for the negative findings in the rapidly fatal cases reported by Wilson and Chowning,[70] Le Count [71] and Wolbach.[72]

Gross examination of the brain generally reveals more or less marked vascular congestion and infection of the meningeal vessels. In 4 of 18 cases reported by Lillie [73] there were numerous punctate hemorrhages in the parenchyma.

The microscopic lesions described by Lillie [73] were of a focal character and of three general types. There was, first, lymphocytic cellular exudation in the sheaths of the smaller vessels, associated with proliferative endarteritis and occasional perivascular bleeding. Second there were perivascular and parenchymal glial nodules. Many of the nodules contained a necrotic center forming a small granuloma. Third, there was actual vascular thrombosis, associated with demyelination or necrosis of the perivascular parenchyma. Lesions of the third type were most numerous in the white matter and in the region of the brain stem. Materials from the cases reported by Pinkerton and Maxcy [74] and by Harris [75] were examined by Lillie, and the lesions were the same as noted in his series. Florman and Hofkenschiel [76] observed, besides the microinfarcts and glial nodules, many small perivascular accumulations

63. Rumreich, A.; Dyer, R. E., and Badger, L. F.: Pub. Health Rep. **46**:470, 1931.

64. Toomey, N.: Ann. Int. Med. **5**:1296, 1932.

65. Baker, G. E.: Ann. Int. Med. **17**:247, 1942.

66. Smith, E. B., and Reinhard, E. H.: J. Missouri M. A. **40**:166, 1943.

67. Bennett, T. B.: M. Bull. Vet. Admin. **17**:425, 1941.

68. Palatucci, O. A., and Maragoni, B. A.: Bull. U. S. Army M. Dept., 1944, no. 79, p. 116.

69. Lillie, R. D.: Pub. Health Rep. **46**:2840, 1931.

70. Wilson, L. B., and Chowning, W. M.: J. Infect. Dis. **1**:31, 1904.

71. LeCount, E. R.: J. Infect. Dis. **8**:421, 1911.

72. Wolbach, S. B.: J. M. Research **41**:1, 1919.

73. Lillie, R. D.: Pub. Health Rep. **46**:2840, 1931.

74. Pinkerton, H., and Maxcy, K. F.: Am. J. Path. **7**:95, 1931.

75. Harris, P. N.: Am. J. Path. **9**:91, 1933.

76. Florman, A. L., and Hofkenschiel, J.: Bull. Johns Hopkins Hosp. **66**:601, 1936.

of monocytes. Hassin [77] reported an aseptic type of meningitis and subarachnoid collections of lipoid-containing histiocytes. The ganglion cells throughout the brain exhibited slight swelling and chromatolysis. Lipoid granules were observed in the cytoplasm of the ganglion cells, in oligodendrocytes and ependymal cells and in the adventitial spaces about the small blood vessels. A similar fatty degeneration of the ganglion cells was reported by Scheinker.[78]

The pathologic changes observed in experimental Rocky Mountain spotted fever are similar to those found in the human disease (Lillie [69]).

São Paulo exanthematous typhus of Brazil is both clinically and pathologically identical with North American Rocky Mountain spotted fever (Dias and Martins [79]; de Toledo Piza, Meyer and Salles Gomes [80]). Topping, Heilig and Naidu [81] have stated that tick typhus in India resembles the North American disease. Fièvre boutonneuse is a somewhat milder disease, and no pathologic studies of human cases are available.

Tsutsugamushi Fever.—Tsutsugamushi fever (scrub typhus) is caused by Rickettsia tsutsugamushi (Rickettsia nipponica, Rickettsia orientalis) and is transmitted to man by several species of larval mites of the genus Trombicula. It has been recorded from Japan, Formosa and Korea, the Malay States, the Philippines, Australia, India, Indo-China, Sumatra and from many of the islands in the South Pacific.

Clinically it resembles other rickettsial diseases. Symptoms ascribable to involvement of the nervous system form an important part of the clinical picture (Ahlm and Lipschutz [82]; Lewthwaite and Savoor [83]; Lipman and co-workers [84]; Reynes and Richard [85]; Heaslip [86]). Hay [87] in a study of 50 patients found early and intense headache in 45, while all the patients suffered from drowsiness and extreme prostration.

The changes in the nervous system were first adequately examined by Lewthwaite,[88] in 1936. Gross examination in 12 cases showed sub-

77. Hassin, G. B.: Arch. Neurol. & Psychiat. **44**:1290, 1940.

78. Scheinker, I. M.: Arch. Path. **35**:583, 1943.

79. Dias, E., and Martins, A. V.: Am. J. Trop. Med. **19**:103, 1939.

80. de Toledo Piza, J.; Meyer, J. R., and Salles Gomes, L.: Typho exanthematico de São Paulo, São Paulo, Sociedade Impressora Paulista, 1932.

81. Topping, N. H.; Heilig, R., and Naidu, V. R.: Pub. Health Rep. **58**:1208, 1943.

82. Ahlm, C. E., and Lipschutz, J.: J. A. M. A. **124**:1095, 1944.

83. Lewthwaite, R., and Savoor, S. R.: Lancet **1**:255, 1940.

84. Lipman, B. L.; Byron, R. A., and Casey, A. V.: Bull. U. S. Army M. Dept., 1944, no. 72, 63.

85. Reynes, V., and Richard, J.: Bull. Soc. path. exot. **33**:70, 1940.

86. Heaslip, W. G.: M. J. Australia **1**:380, 1941.

87. Hay, C. P.: J. Roy. Nav. M. Serv. **30**:127, 1944.

88. Lewthwaite, R.: J. Path. & Bact. **42**:23, 1936.

dural hemorrhage in 2 and congestion of the brain surface in 6. In 7 cases tissues were examined microscopically. The findings were meager, but the lesions resembled those seen in Rocky Mountain spotted fever and in typhus. In some cases there were few lesions visible, and in 2 cases no diagnostic changes were observed. The most frequent lesion was perivascular proliferation of neuroglia, which in some areas actually formed tiny nodules. The larger nodules also contained lymphoid elements, pyknotic fragments of nuclei and degenerate erythrocytes. Many vessels showed proliferative endarteritis with heavy pigmentation of the endothelial cells. Clumps of rickettsias were observed in the cytoplasm of the endothelial cells in 5 of the 7 cases. Small perivascular hemorrhages were frequent. The changes were most numerous in the pons and the medulla and least numerous in the cerebellum. Kouwenaar [89] in 1940 found many small foci of round cell infiltration around the smaller vessels, with the infiltrates penetrating into the walls of the vessels, resulting in necrosis of the intima and subsequent thrombosis. Corbett [90] in 1943 described the brain changes in 4 cases. He considered the perivascular proliferation of glial cells and the lymphocytic infiltration as the most characteristic lesion in this disease. The lesions were most readily observed in the pons but were found also in the cerebrum, the cerebellum and the medulla.

Kouwenaar and Wolff [91] in a description of mite fever in the guinea pig noted lesions of the brain similar to those found in one half of their cases of typhus.

Q Fever.—Q fever (Queensland fever, quadrilateral fever) was first described from Australia in 1937 by Derrick [92] and from the United States in 1938 by Dyer.[93] It is caused by Rickettsia burneti, which is transmitted from the natural animal reservoir to man by infected ticks. Although in this disease there are symptoms which suggest involvement of the central nervous system, there is at present no pathologic evidence in man to substantiate such an impression. Most of the studies of human Q fever are limited to the pulmonary changes. Lillie [94] studied the nervous system changes in guinea pigs infected with rickettsias of Q fever. He observed perivascular lymphocytic infiltration and proliferation of the vascular endothelium, as well as a few glial nodules. He stated that "compared with endemic and European typhus, or even with Rocky Mountain spotted fever, focal brain and cord lesions in the guinea pig are strikingly infrequent."

89. Kouwenaar, W.: Geneesk. tijdschr. v. Nederl.-Indië **80**:1119, 1940.
90. Corbett, A. J.: Bull. U. S. Army M. Dept., 1943, no. 70, p. 34.
91. Kouwenaar, W., and Wolff, J. W.: J. Infect. Dis. **55**:315, 1934.
92. Derrick, E. H.: M. J. Australia **2**:281, 1937.
93. Dyer, R. E.: J. A. M. A. **122**:331, 1943.
94. Lillie, R. D.: Pub. Health Rep. **57**:296, 1942.

Trench Fever.—Trench fever (Wolhynian fever, five day fever) is a specific louse-borne disease which seems to be associated with war. The exact nature of the etiologic agent has not been determined, although there is considerable evidence that it may prove to be a rickettsia. Although many reports indicate that the central nervous system is involved, most patients recover and no pathologic studies of the affected nervous system are available at present.

BACTERIAL DISEASES

Bartonellosis.—Bartonellosis, Oroya fever or verruga peruana is a disease peculiar to Peru, although recently cases have been reported in Colombia and in Ecuador (Camargo [95]). It has been recognized for many years and has appeared in two clinical forms, namely, as a severe, highly fatal febrile anemia (Oroya fever) and as a cutaneous eruption (verruga peruana). For many years it was doubtful whether the aforenamed two conditions were related. In 1885 Daniel A. Carrión vaccinated himself with blood from a Peruvian wart, and in twenty-one days Oroya fever developed, from which he died, thus proving the interrelationship of the two conditions. The causative organism was isolated in 1902 by Barton.[96] In 1913 it was assigned to the genus Bartonella and is considered to be midway between a rickettsia and a bacillus.

Carrión [97] first called attention to the frequent involvement of the nervous system. The neurologic manifestations are most variable, since the organism is capable of affecting all parts of the nervous system. Characteristically, various clinical forms of neurobartonellosis have been described, namely: (1) hypertensive, (2) meningeal, (3) convulsive, (4) apoplectiform, (5) extrapyramidal and (6) mental.

The histologic nature of the cerebral lesions has been described in detail by Mackehenie and Alzamora [98] and by Lastres.[99] The chief involvement is vascular, implicating primarily the capillaries, the precapillaries and the small venules. Apparently the first lesions involve the endothelial cells, which are invaded by the organisms and undergo swelling and proliferation, producing partial or even complete vascular occlusion. Many of these involved endothelial cells break off into the vessel, filling the lumen with parasitized cells. This produces an occlusive thrombus, with the result that focal areas of tissue degeneration develop, with destruction of many of the neighboring cell elements. The nerve cells adjacent to these occluded vessels reveal many acute

95. Camargo, L. P.: Fac. de med., Bogotá **9**:160, 1940.

96. Barton, A. L.: Crón. méd., Lima **18**:193, 1902.

97. Carrión, D. A., cited by Lastres.[99]

98. Mackehenie, D., and Alzamora, V. V.: J. neuro-psiquiát. panam. **2**:166, 1940.

99. Lastres, J. B.: Rev. méd. peruana **6**:1690, 1934.

changes, consisting of swelling, chromatolysis and even fragmentation and disappearance of nerve cells. Within the white matter there occur foci of necrosis, invaded by fat granule cells. As a result of this ischemia, the vessels themselves undergo weakening, with red cells and some leukocytes escaping into the perivascular spaces and even beyond into the injured brain tissue. Another very prominent alteration consists in the appearance of tiny granulomas adjacent to many of the involved vessels. These granulomas result from focal adventitial proliferation associated with rapid multiplication of local histiocytes and monocytes to produce a nodule composed chiefly of mesenchymal elements. They also contain scattered lymphocytes, plasma cells and many multinucleated elements. They are observed chiefly in the gray substance of the brain (Lastres [100]) and vary in frequency from case to case. A few scattered vessels contain no real granulomas but tiny collections of round cells within their adventitial layers or within the perivascular spaces. Around many of the ischemic foci within the white matter appear tiny foci of glial proliferation producing glial rosettes. At no time is this glial response of a diffuse nature.

In the more chronic process the granulomas undergo fibrosis, which leaves fibrous tissue scars adjacent to the vessels.

The lesions in neurobartonellosis are extremely widespread and have been observed within all regions of the nervous system. Mackehenie and Alzamora [98] in a thorough pathologic study observed extensive changes not only within the brain but also within the sympathetic nervous system, the peripheral nerves and the pituitary gland. The brachial plexus revealed the typical vascular occlusions and granulomas involving the perineural vessels. There was monocytic exudation among the nerve fasciculi. The pituitary gland showed blood vessels occluded by parasitized elements, with zones of necrosis and disorganization of the glandular acini resulting. Granulomas were present within both the anterior and the posterior lobe of this gland. Bartonellas have been observed within the dilated vessels of the choroid plexus as well as within the astrocytes and the microgliocytes (Lastres [100]; Monge and Mackehenie [101]).

Monge and Mackehenie in a series of cases observed old clotted blood covering the hemispheres in 1 case and numerous punctate hemorrhages in 1. In all cases there was revealed marked vascular congestion on gross inspection. Histologically the typical endothelial proliferation, occlusive thrombosis and granulomatous formations were revealed in all the brains.

Bacillary Dysentery.—Bacillary dysentery is an acute infectious disease caused by Bacillus dysenteriae and resulting primarily in gastro-

100. Lastres, J. B.: América clin. **5**:11, 1943.
101. Monge, C., and Mackehenie, D.: Rev. méd. peruana **4**:523, 1932.

intestinal symptoms. The disease is acute in onset and terminates either abruptly in death after four to six days or more slowly in gradual recovery. The disease is more virulent in the tropics, where it appears in epidemic form (India, Indo-China, Japan, Java, Northern Brazil, Egypt, Palestine and other areas). In the United States and northern Europe the outbreaks are small and often sporadic, being most frequent in hospitals for patients with mental diseases and in orphanages and among troops during war. The outbreaks seem influenced primarily by sanitation rather than by geographic distribution since the disease is spread chiefly by contamined food and drink or by carriers convalescing from the disease. The organism was first isolated in 1898, by Shiga,[102] in Japan. Since then it has been recognized that B. dysenteriae comprises a large group of micro-organisms (Flexner[103]; Strong and Musgrave[104]; Kruse[105]; Hiss and Russell[106]). At present all dysentery bacilli are divided into two large groups, namely, those that produce no acid on mannite sugar and no indole (Shiga-Kruse type) and those that do produce acid on mannite and do form indole (Flexner-Hiss-Strong type).

Dysentery bacilli are usually present in the gastrointestinal tract, where ulceration results. Even in the more severe lesions the organisms remain localized to the bowel, rarely invading the blood stream. Since the bacilli remain so well localized, it is generally believed that the cerebral complications are secondary in type and probably result from toxic circulatory or metabolic factors. The frequency of complications referable to the nervous system varies with the severity of the infection. Zellweger[107] in a study of cases observed such complications in 32.7 per cent, while Gröer[108] reported involvement of the nervous system in 56 per cent of his 50 cases. Symptoms referable to the nervous system occur chiefly in children and are not infrequent in patients with profound acute and chronic dysentery (Zellweger[107]; Alexander and Wu[109]). The clinical picture may be variable. It often resembles that of epidemic encephalitis except that it is preceded or accompanied by a specific type of dysentery. The peripheral nervous system may also be involved. This complication occurs chiefly in adults during the period from the second to the fourth week of illness and may take the form

102. Shiga, K.: Zentralbl. f. Bakt. (Abt. 1) **24**:817, 870 and 913, 1898.
103. Flexner, S.: Bull. Johns Hopkins Hosp. **11**:231, 1900.
104. Strong, R. P., and Musgrave, W. E.: J. A. M. A. **35**:498, 1900.
105. Kruse, W.: Deutsche med. Wchnschr. **26**:637, 1900.
106. Hiss, P. H., and Russell, F. F.: J. M. Research **13**:1, 1904.
107. Zellweger, H.: Praxis **32**:707, 1943.
108. von Gröer, F.: Ztschr. f. Kinderh. **21**:220, 1919.
109. Alexander, L., and Wu, T. T.: Chinese M. J. **48**:1, 1934.

of mononeuritis or peripheral neuritis (Müller-Deham [110]; Singer [111]; Alexander and Wu [109]; Zellweger [107]).

Only scattered reports describing the changes in the nervous system in man or even in experimental animals are available. Probably the most extensive studies have been reported by Alexander and Wu.[112] They studied 16 cases of bacillary dysentery and reported what they called "circulatory and regressive parenchymal changes" but no inflammatory alterations. Sporadic ischemic foci were present throughout the cortical gray matter, involving chiefly the third to fifth cortical layers and located near small vessels. These areas showed deficiency of nerve cells or alteration of numerous neurons of varying degree and type. Some cells were pyknotic, while others had lost their staining properties. Many of these foci were replaced by proliferated glial elements. Many of the neurons unassociated with these ischemic foci were pyknotic and revealed chronic alteration of the nerve cells, even being replaced or surrounded by glial rosettes. With Bielschowsky stains, these cells showed argentophilia with thickening, breaking and crumbling of the intracellular neurofibrillae and argentophilia of the nuclei and the glial cells. The cerebellar cortex revealed damage of the Purkinje cells with formation of glial shrubs along the dendrites and about the damaged cell. In 1 case there was complete ischemic necrosis of the cerebellar cortex with focal destruction of the granular layer and the Purkinje cells. Glial nodules were scattered throughout, unassociated with the ischemic areas. In only 1 case was lymphocytic infiltration observed within the white matter, chiefly of perivascular distribution.

The remaining reports within the literature describe changes much less extensive. Buttenwieser [113] reported a case of Shiga-Kruse dysentery in which death occurred two weeks after the onset of illness. At autopsy numerous ring and ball hemorrhages were observed throughout the cortex, the basal ganglions and the midbrain. Oesterlin [114] in autopsy studies on 11 cases of dysentery observed significant changes in only 1. The patient was a 17 month old child who died following a period of motor unrest, vomiting and bloody diarrhea. At autopsy changes were observed chiefly in the cerebellum. The molecular layer contained an occasional glial nodule. In certain areas there was shrub-like proliferation of glia about the Purkinje cells. A diffuse glial increase was also observed, involving chiefly the granular and inner molecular layers. Spielmeyer [115] was the only investigator to describe

110. Müller-Deham, A.: Wien. med. Wchnschr. **65**:654, 1915.
111. Singer, K.: Monatschr. f. Psychiat. u. Neurol. **41**:245, 1917.
112. Alexander, L., and Wu, T. T.: Arch. Neurol. & Psychiat. **33**:72, 1935.
113. Buttenwieser, S.: München. med. Wchnschr. **76**:1472, 1920.
114. Oesterlin, E.: Ztschr. f. d. ges. Neurol. u. Psychiat. **88**:323, 1924.
115. Spielmeyer, W.: Ztschr. f. d. ges. Neurol. u. Psychiat. **123**:161, 1930.

small necrotic foci around small blood vessels of the white matter in dysentery.

A few reports are available illustrating experimental lesions, chiefly those in rabbits. Lotmar,[116] using the Shiga-Kruse toxin, observed changes involving all parts of the nervous system. The lesions were of two types. Some were focal in nature and consisted of areas of devastation in which all cell elements were degenerated. These foci often were surrounded by a mild glial increase. In certain foci the neurons were only partially destroyed and showed marked granular degeneration. The second type of lesions consisted of "irritative encephalitides." These were comprised of focal collections of cells, chiefly glia cells intermixed with fat granule cells and plasma cells. Vessels in the involved areas showed intensive lymphocytic infiltrations and some endarteritis. These two types of lesions occurred independently of each other in different animals. Guggisberg,[117] using 20 rabbits and intravenous injections, found no focal lesions and only diffuse neuronal involvement, consisting of tigrolysis, nuclear disintegration, glial increase, but no vessel changes. Tupa [118] and Karasawa [119] observed alterations, limited chiefly to the spinal cord. The lesions seemed to localize within the cervical cord and bulb and appeared less commonly within the lumbar regions. Isolated lesions were observed by Tupa within the thalamus, the olfactory lobes and the cerebellum. In the cord the gray matter was chiefly involved. Hyperemia and hemorrhages were common. The anterior horn cells were swollen, chromatolytic and even vacuolated. There was only mild glial increase.

Cholera.—Cholera is an infectious disease caused by Vibrio comma and involves primarily the intestinal tract. These vibrios are spread chiefly by contaminated food and water. The disease has been endemic in many parts of the world (India, Asia and the Far East). Six worldwide pandemics have been traced to India, and all except the first spread throughout Europe, producing tremendous devastation. Most of the studies in the complications referable to the central nervous system are based on studies of cases resulting during the outbreak of 1891, which produced over a million deaths in Russia alone.

Encephalic complications are almost never mentioned in the usual descriptions of cholera, in spite of their frequency during the larger pandemics. Often the clinical symptoms referable to the nervous system are so mild as to be entirely overlooked. The patient's face becomes

116. Lotmar, F.: Ztschr. f. d. ges. Neurol. u. Psychiat. **8**:345, 1911.

117. Guggisberg, H.: Arb. a. d. Inst. z. Erforsch. d. Infektions-krankh. in Bern, 1908, p. 51.

118. Tupa, A.: Compt. rend. Soc. de biol. **92**:1141, 1925.

119. Karasawa, M.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **6**:390, 1910.

drawn and haggard; sleep becomes disturbed and thinking difficult. In the more severe type there may occur vertigo, headaches, restlessness, convulsions, apathy progressing to stupor or severe delirium (Gavino [120]; Weber and Ranke [121]; Delasiauve [122]; Burq [123]; Obregia and Pitulesco [124]; Kraepelin [125]; Mesnet [126]; Ball [127]).

Only scattered studies are available on the changes in the central nervous system affected by cholera, and many of these are in the earlier Russian literature, which was not available to us. Most of the studies appeared following the European pandemic of 1891, particularly in the German and the Russian literature. In 1873 Iwanowsky [128] reported diffuse cortical changes. The cortical neurons showed swelling and some tinctorial loss. Scattered cells became smaller, and each was surrounded by an enlarged pericellular space. The blood vessels of the cortex were congested, and their endothelium was swollen and often filled with fat. Changes of the types noted, involving chiefly the cortical neurons and the vessels, appear to be fairly constant and have been recorded repeatedly. Tizzoni and Cattani [129] in 1888 studied the brains of 54 persons dying in Bologna, Italy, in 1886 and found changes similar to those of Iwanowsky. They also described some cellular chromatolysis and dilatation of the perivascular spaces. They were able to isolate V. comma from the spinal fluid of 2 of their patients. In 1894 Popoff [130] reported his observations on 2 patients dying in Warsaw, Poland. Again the most striking changes occurred within the cortical neurons, which were diffusely and often severely injured. Most of the cells showed swelling, chromatolysis and eccentricity of the nuclei. In many, however, even fragmentation had occurred. The nuclei showed no changes in spite of the severity of the cell damage. The blood vessels were congested and showed marked endothelial swelling and proliferation. Some of the larger vessels contained small yellowish masses both within their walls and within their perivascular spaces. Popoff reported a diffuse glial increase throughout both the gray and the white matter, particularly in the patients whose disease had become chronic. Tschistowitch [131]

120. Gavino, C.: J. Philippine Islands M. A. **7**:3, 1927.
121. Weber and Ranke: Lancet **2**:344, 1853.
122. Delasiauve, M.: Ann. méd.-psychol. **13**:331, 1849.
123. Burq, M. V.: Gaz. méd. de France **5**:82, 1850.
124. Obregia and Pitulesco: Encéphale **9**:393, 1914.
125. Kraepelin, E.: Arch. f. Psychiat. **12**:322, 1882.
126. Mesnet, E.: Ann. méd.-psychol. **30**:317, 1866.
127. Ball, M. B.: Encéphale **1**:30, 1885.
128. Iwanowsky, N., cited by Tschistowitch.[131]
129. Tizzoni, G., and Cattani, G.: Beitr. z. path. Anat. u. z. allg. Path. **3**:191, 1888.
130. Popoff, N. M.: Virchows Arch. f. path. Anat. **136**:42, 1894.
131. Tschistowitch, T. J.: Virchows Arch. f. path. Anat. (supp.) **144**:40, 1896.

in 1896 reported on 21 cases, and his findings were similar to those of Popoff although he noted no glial increase. The nerve cells in Tschistowitch's cases were often severely implicated even to the stage of vacuolation. In very severe long-standing cholera the cell damage was often so severe that large areas of cortex were filled with vacuolated, fragmented, granular cytoplasmic masses. The motor cortex and the cerebellum were uninvolved. The vascular congestion and endothelial proliferation were also often marked, with some of the vessels being surrounded by perivascular bleeding. In an occasional subacute case of the form of the disease the author observed markedly widened perivascular spaces, often containing leukocytes.

One of the most recent studies of cases of cholera was published in 1922 by Pines.[132] In these cases, also, the chief alterations were limited to the cortex with diffuse destruction of the nerve cells. There appeared to be progressive enlargement of the pericellular and perivascular spaces. These spaces continued to enlarge so that in the more chronic processes the entire cortex was filled with numerous tiny cavities, some of which were filled with amorphous masses or degenerated nerve cells. The author observed no changes within the white matter or the cerebellum.

The spinal cord also becomes involved in Asiatic cholera. Popoff [130] observed some changes within the anterior horn cells, which were at times vacuolated and filled with yellowish granules. Scattered cells contained more than one nucleus. In the white matter there occurred swelling of the axis-cylinders, particularly in the lateral columns. Michailow [133] studied the cord in 2 fatal cases of cholera and observed scattered clumps of V. comma in selected areas of the cord. The anterior horn cells in the regions where the micro-organisms were found showed complete chromatolysis, while the neurons at other levels were uninvolved. In 1912 Michailow [134] again reported on the cord changes in 8 cases of cholera. In the acute illness no changes were visible. The most constant alteration consisted of myelin degeneration around the periphery of the cord. In 2 cases some scattered demyelination had occurred also within the lateral and the posterior columns. The author felt that the latter changes were secondary to axonal degeneration following damage of cortical nerve cells.

The etiologic explanation of these changes in the central nervous system has been the subject of considerable discussion. Both Michailow [134] and Tizzoni and Cattani [129] isolated V. comma from the spinal fluid and from cord tissue and held that the changes were produced by the organisms themselves and that these reached the nervous system

132. Pines, I. L.: Arch. f. Psychiat. **66**:796, 1922.
133. Michailow, S.: Zentralbl. f. Bakt. (Abt. 1) **62**:545, 1912.
134. Michailow, S.: Zentralbl. f. Bakt. (Abt. 1) **50**:296, 1909.

by way of the blood stream. This theory receives some support from the fact that V. comma has been demonstrated in the blood by many investigators. On the other hand, bacteremia is rare in cholera, and the organism does have a tendency to remain localized to the intestine. Most investigators (e. g. Tschistowitch [131] and Stitts and Strong [135]) have felt that V. comma produces a powerful endotoxin, which is set free when the vibrio undergoes disintegration. It is the absorption of this toxin that produces the severe complications referable to the nervous system. Finally, Kraepelin [125] believed that the toxin of cholera rarely reaches the brain. He thought that most of the encephalic involvement was secondary to anoxia, which was produced by the severe diarrhea and dehydration with subsequent high concentration of blood.

Bubonic Plague.—Bubonic plague is a specific infectious disease affecting man and some of the lower animals. It is produced by a short round rod, Bacillus or Pasteurella pestis. The classic form of this disease, as the name indicates, manifests itself after an initial period of generalized symptoms by painful swellings with effusions into the lymphatic glandular tissues chiefly of groin, armpit and neck, resulting in the appearance of the classic buboes. This disease may manifest itself in a pneumonic and a septicemic form, in which the buboes do not occur or, if they do, appear only as a late manifestation of the disease. Other forms have been described, such as carbuncular, intestinal and cerebral forms (Simpson,[136] Choksy [137]). Plague may manifest itself in sporadic, epidemic and pandemic modes and probably has caused more deaths in the world than any other human illness.

This disease is one of great antiquity and was well recognized in Syria and its vicinity three thousand years ago. The disease prevailed in endemic and epidemic modes within the countries on the southern and eastern shores of the Mediterranean (Libya, Egypt, Syria), spreading periodically over most of Europe (sixth, eleventh and fourteenth centuries.) In the United States the first infection reached San Francisco from Honolulu, Hawaiian Islands, in 1900. By 1907 it had spread over the entire state (Lloyd [138]; Kellogg [139]). In 1924, 32 cases of plague were identified in Los Angeles, and by 1942 the disease had appeared in the states of Nevada, Utah, North Dakota and Wyoming. According to Lloyd, the endemic center in the United States was the California ground squirrel. He surmised that plague would remain a menace to inhabitants throughout the world indefinitely.

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135. Strong, R. P.: Stitt's Diagnosis, Prevention and Treatment of Tropical Diseases, Philadelphia, The Blakiston Company, 1943, vol. 1, p. 602.
136. Simpson, W. J.: A Treatise on Plague, Cambridge, University Press, 1905.
137. Choksy, K. B.: Am. J. M. Sc. **138**:351, 1909.
138. Lloyd, B. J.: J. A. M. A. **85**:729, 1925.
139. Kellogg, W. H.: Am. J. Pub. Health **10**:599, 1920.

The causative organism of plague was first described by Yersin, in 1894. It is a short round or oval rod with bipolar staining. In the living body the bacilli are abundant in buboes, as shown by smears, in the sputum if the infection is pneumonic and in the blood if septicemia develops. At autopsy the organisms can be obtained from many organs, including the brain, and from the spinal fluid (França [140]).

Next to the buboes and the pneumonic lesions, the encephalic changes comprise the most common disturbance. Since the causative organism is found within the brain, the involvement of this organ is a primary one and constitutes a primary encephalitis. Symptoms of such involvement are therefore extremely common in all forms of plague and frequently constitute the predominating symptomatic aspect. Usually these symptoms are of a diffuse rather than a focal nature, indicating a widespread involvement of the nervous system. Cerebral complications occur most frequently in the bubonic form of the disease, in which the course is a little more prolonged.

The chief neurologic complaints are headaches and vertigo, which increase in severity. As the disease progresses, the severe toxemia affects the intellect more and more, producing mental dulness, confusion and delirium. Terminally the patient shows increasing lethargy and coma. In the septicemic form, because of the overwhelming infection of the blood stream, the cerebral symptoms develop with great rapidity, and the course of the illness may be short.

Most of the general symptoms no doubt are due to the extreme toxemia occurring in plague. Since the bacillus of plague actually localizes within the meninges or the brain substance, one would expect focal symptoms to accompany the more general involvement already described. The most characteristic of these focal phenomena are ataxic gait and incoordination of speech (Wu Lien-Teh [141]; Simpson [136]).

It is surprising that there have been so few detailed pathologic studies of plague reported, particularly since it is generally agreed that the nervous system is one of the organs chiefly involved. Since this illness is largely a disease of the lymphatic and the vascular system through which the bacilli of the disease and their toxins are brought in contact with nearly every part of the body, it would be expected that the chief lesions within the brain would be centered about the vessels. Edema, congestion and hemorrhage within the meninges and the brain tissue have been frequently reported (Wu Lien-Teh [141]; Stitts and Strong [142]). Stitts and Strong described petechiae as occurring primar-

140. França, C.: Névraxe **1**:321, 1900.

141. Wu Lien-Teh.: A Treatise of Pneumonic Plague, Publication of the League of Nations, Geneva, Switzerland, 1926, vol. 3.

142. Strong,[135] p. 688.

ily within the meninges, the mesencephalon and the medulla. Calmette and Salimbeni [143] and Crowell [144] described meningitis primarily in their fatal cases. In both of Crowell's cases at autopsy the cerebrospinal fluid was turpid and the subarachnoid space and the ventricles were covered with a thick, stringy yellow pus which was loaded with plague bacilli. Calmette and Salimbeni described 2 fatal cases, 1 of meningitis and 1 of meningoencephalitis. In the latter the gray matter was congested and edematous, while the subarachnoid space contained an exudate yielding plague bacilli on culture.

By far the most complete autopsy studies on plague have been reported by Franca [140] and Nepveu.[145] Nepveu described only mild meningitis, with many of the venules thrombosed and containing clumps of plague bacilli. The cortical vessels were frequently thrombosed and filled with bacilli. Many of the vessels were surrounded by leukocytes, this perivascular involvement decreasing within the deeper cortical layers. The cortical neurons were extensively damaged. Many were surrounded by leukocytes. These showed chromatolysis, fragmentation of the cell processes, disintegration of the cell body and destruction of the axons entering the cell. Many damaged nerve cells were unassociated with a leukocytic reaction. The damaged cells were swollen, pale, vacuolated or granular. Their nucleoli were often displaced peripherally. These neuronal changes were irregular, involved scattered cells within the cortex and were present in varying degrees of severity. The neuroglia was moderately increased. França reviewed 11 cases and found bacilli in all but 3. The bacilli were observed most frequently within the thrombosed vessels but could also be identified free within the white matter or within the cortical neurons and the adjacent tissues. The most severe neuronal alterations occurred within the cerebral cortex, the cerebellum and the medulla. The nerve cells were swollen, stained lightly and showed peripheral chromatolysis. In the more prolonged cases, the cell body was vacuolated and even fragmented, the nuclei pyknotic and nucleoli eccentrically placed. In 3 cases the most severe cellular damage appeared within the motor cortex, but in most cases the involvement was patchy, with many altered cells occurring among entirely unchanged elements. Babes [146] studied the effect of plague toxin on the spinal cord of the rabbit. With large doses, the animals died within two days and showed swelling, vacuolation and fragmentation of the processes of cells within the anterior horns. When smaller doses of toxin were administered, the rabbits lived for six days and revealed

143. Calmette, A., and Salimbeni, A. T.: Ann. Inst. Pasteur **12**:865, 1899.
144. Crowell, B. C.: Philippine J. Sc. **10**:249, 1915.
145. Nepveu, M. G.: Compt. rend. Soc. de biol. **49**:863, 1897.
146. Babes, V.: Klin. Wchnschr. **35**:36, 1898.

hemorrhages and leukocytic infiltration within the anterior horns. The cells of the anterior horns were only mildly altered. In the more chronic illness, the round cell infiltration increased, while the neuronal damage diminished in severity.

Melioidosis.—Melioidosis is a tropical disease which is localized to the extreme Orient, being found chiefly in the Malay States, Netherlands Indies, Ceylon and Indo-China. It produces in man a disease resembling glanders. This illness was first recognized by Whitmore and Krishnaswamy,[147] in 1912, while they were studying cadavers of vagabonds. In the following years Whitmore isolated the bacillus, which he named Bacillus pseudomallei and which has been renamed Bacillus whitmori. By 1933 the numbers of cases of melioidosis observed in the Orient totaled 95, with numerous cases occurring in Europe.

Pathologically the characteristic lesion consists of small yellowish caseous nodules which begin as focal collections of leukocytes and increase in size. The lesions appear as military granulomas with necrotic centers. They have been reported in all organs except the brain. However, the organism has been found in the blood of patients with the septicemic form of the disease, and the clinical picture of the involvement of the central nervous system certainly suggests the probablity of cerebral involvement.

Leprosy.—Leprosy is a well known infectious disease which has been recognized in man since great antiquity. It has been endemic in Egypt, Persia, India and much of the rest of the Orient since ancient days. According to McCoy [148] there are 400 to 500 persons with the disease in active form in the United States. In South America there are some 30,000, particularly in Brazil.

This disease probably owes its spread to contacts occurring over a long period with those having active lesions, but the exact mode of spread or portal of entry of the infective organism is not known. There are two well recognized types of the disease. One is the nodular or cutaneous variety, characterized by granulomatous proliferation within the skin and the subcutaneous tissues forming the so-called leproma. This consists of a mass of so-called leprosy cells or foam cells which are often filled with leprosy bacilli and intermixed with various types of connective tissue. The second type is known as the maculoanesthetic or neural type and is characterized by flat thickenings within the covered regions of the body associated with areas of anesthesia within these involved regions The nerves become thickened, and muscular palsies and atrophies develop, associated with ulcerations, contractures and severe mutilations secondary to the trophic changes.

147. Whitmore, A., and Krishnaswamy, C. S.: Indian M. Gaz. **47**:262, 1912.
148. McCoy, G. W.: Arch. Dermat. & Syph. **37**:169, 1938.

Bacillus leprae (Mycobacterium leprae) has been accepted as the cause of human leprosy and is present in large numbers in the characteristic lesions throughout the body as well as within the peripheral and even the central nervous system. There is some question whether the organisms occur within the circulating blood, but Stitts and Strong expressed the belief they do and probably spread in this way from organ to organ.

Occasionally during the course of the disease certain special symptoms may develop which suggest a more specific involvement of the nervous system. Many patients, especially at the beginning of their illness, show a tendency toward somnolence and complain of severe headaches. Often this somnolence is associated with frequent nightmares of a terrorizing nature, visual hallucinations and an agonizing sensation of stuffiness. Motor and sensory manifestations are chiefly referable to involvement of the peripheral nervous system. Mental symptoms have been reported in many cases of leprosy (Bodros [149]; Jakob and Meggendorfer [150]; de Beurmann and co-workers [151]; Muir [152]; Jones and Pearson [153]; Swerbejew [154]). All forms of psychoses have been observed.

Naturally the appearance of an organic psychosis in a patient with leprosy, as well as the appearance of the bacilli in the circulation, make one consider the possibility of actual encephalitis with definite changes within the central nervous system. Reports of brain changes in this disease are surprisingly few. Many authors who have studied the brain in leprosy have expressed the opinion that no definite changes occur (Thoma [155]; Neisser [156]; Leloir [157]; Gerlach [158]; Rikli [159]). However, the bacilli of leprosy have been reported within the intracranial cavity in isolated cases. Doutrelepont and Wolters [160] observed them within the perivascular spaces of the pia, while Brutzer [161] found them in the

149. Bodros, P.: Ann. méd.-psychol. **1**:278, 1912.

150. Jakob, A., and Meggendorfer, F.: Arch. f. Dermat. u. Syph. **130**:367, 1921.

151. de Beurmann; Roubinovitch, and Gourgerot: Rev. neurol. **14**:292, 1906.

152. Muir, E.: Leprosy Rev. **10**:114, 1939.

153. Jones, R., and Pearson, R.: Lancet **179**:728, 1910.

154. Swerbejew, N.: Arch. f. Psychiat. **108**:572, 1938.

155. Thoma, R.: Virchows Arch. f. path. Anat. **57**:455, 1873.

156. Neisser, A.: Virchows Arch. f. path. Anat. **84**:514, 1881.

157. Leloir, H.: Arch. de physiol. **8**:391, 1881.

158. Gerlach, W.: Virchows Arch. f. path. Anat. **125**:126, 1891.

159. Rikli, A.: Virchows Arch. f. path. Anat. **129**:110, 1892.

160. Doutrelepont and Wolters, M.: Arch. f. Dermat. u. Syph. **34**:80, 1896.

161. Brutzer, C.: Dermat. Ztschr. **5**:750, 1898.

dura over the hypophysis. Lie [162] in a careful study of 5 cases observed the bacilli within the nuclei of the medulla, particularly within the facial nerve, with no clinical evidence of involvement. Lie surmised that in nodular leprosy the entire nervous system is heavily invaded but that the brain is immune against the bacilli and thus the organisms are unable to grow.

The most complete studies on the brain changes in leprosy have been published by Dwijkoff,[163] Vilde,[164] Stahlberg [165] and Jacob and Meggendorfer.[150] In most cases the changes were diffuse, involving large areas of the brain but being most marked within the basal ganglions, the midbrain and the medulla. The nerve cells showed patchy chromatolysis, with some cells having undergone complete tigrolysis, some vacuolation and definite nuclear changes with pyknosis and irregularity of nuclear outline. Dwijkoff observed some fatty degeneration of the ganglion cells. Both Vilde and Stahlberg observed cerebellar changes. The former reported definite reduction in the Purkinje cells, while the latter observed the most severe changes within the molecular layer. The vessels throughout the brain revealed marked congestion. Many of the smaller ones showed marked endothelial increase, with fatty changes involving both the endothelium and the medial elements (Dwijkoff; Vilde). Stahlberg observed some widening of the perivascular spaces but no demyelination. The reaction on the part of the glia was most variable. Stahlberg reported the glial elements as also showing degenerative changes, while Vilde observed proliferative glial changes with the production of glial rosettes. Actual presence of inflammatory elements was observed only rarely. Dwijkoff in his case observed a few vessels within the corpus striatum infiltrated with leukocytes, while Vilde reported scattered vessels within the cerebral hemispheres and the medulla encircled by perivascular lymphocytes. In 2 cases this infiltration was quite pronounced especially within the medulla and the midbrain. Vilde also observed some scattered necrotic foci with secondary glial increase. Jakob and Meggendorfer described the cerebral changes in a 25 year old man who died of leprosy. The brain contained a softened area in the right frontoparietal region, which histologically showed chronic degeneration of the parenchyma with fat granule cell invasion and glial increase. The neurons within this area were destroyed. The entire brain, however, revealed scattered changes within the nerve cells of both an acute and a chronic type. Many ganglion cells were swollen and partially chromatolytic. Some of these elements were vacuolated and surrounded by

162. Lie, H. P.: Acta path. et microbiol. Scandinav. **7**:32, 1930.
163. Dwijkoff, P. P.: Frankfurt. Ztschr. f. Path. **40**:185, 1930.
164. Vilde, J.: Ztschr. f. d. ges. Neurol. u. Psychiat. **133**:119, 1931.
165. Stahlberg, H.: Arch. f. Psychiat. **41**:596, 1906.

ameboid cells. A few nerve cells were shrunken and pyknotic. Within the white matter there was diffuse glial increase.

The spinal cord is much more frequently involved in leprosy, although the changes reported have been variable. The most constant alteration is that of the ganglion cells, chiefly those of the anterior horns. These cells were frequently filled with leprosy bacilli (Uhlenhuth and Westphal [166]; Shaw [167]). Chassiotis [168] expressed the opinion that the bacilli were not situated within the nerve cells but appeared, both in the gray and in the white matter, as oval bodies filled with bacilli and covered by a membrane. The earliest neuronal change within the spinal cord was a tinctorial loss, particularly around the bacilli. The affected cell became vacuolated to the point of destruction of the entire cell body. Next to the neuronal damage, one of the most consistent changes in the cord was focal degeneration of the dorsal columns (Lie [162]; Looft [169]; Samgin [170]; Jeanselme and Marie [171]) occasionally associated with involvement of Clarke's columns (Jeanselme and Marie [171]; Tschirjew [172]). In the degenerated areas, axons were only rarely seen, and the posterior rootlets often were atrophic. Etiologically this damage of the cord has been subjected to much speculation. Lie surmised that the changes were secondary to the peripheral neuritic changes and not due to primary involvement by the leprosy bacilli or its toxin. Central cavitation of the spinal cord has also been reported, but in many of the cases true syringomyelia has eventuated rather than leprosy of the cord. Pestana and Bettencourt,[173] however, observed a patient with a syringomyelic syndrome who at death showed lepra bacilli within the cord cavity. Leprosy frequently attacks the sympathetic ganglions and characteristic changes have been reported by many authors (Sudakewitsch [174]; Ermakóva [175]). The ganglion cells showed marked vacuolation, with many leprosy bacilli distributed between the vacuoles in the remnants of the cytoplasm. Bacilli were present within the cell capsule. The nuclei of the vacuolated cells were frequently pyknotic or entirely absent.

Changes within the peripheral nerves have been well described, and the observations require no repetition at this time.

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166. Uhlenhuth and Westphal, A.: Zentralbl. f. Bakt. (Abt. 1) **29**:231, 1901.
167. Shaw, J. D.: Brooklyn M. J. **1**:14, 1888.
168. Chassiotis: Monatsh. f. prakt. Dermat. **6**:1039, 1887.
169. Looft, C.: Virchows Arch. f. path. Anat. **128**:215, 1892.
170. Samgin: Deutsche med. Wchnschr. **24**:475, 1898.
171. Jeanselme, E., and Marie, P.: Rev. neurol. **6**:751, 1898.
172. Tschirjew, S.: Arch. de physiol. **6**:614, 1879.
173. Pestana, C., and Bettencourt, A.: Zentralbl. f. Bakt. (Abt. 1) **19**:698, 1896.
174. Sudakewitsch, J. J.: Zentralbl. f. Chir. **12**:567, 1885.
175. Ermakóva, N.: Internat. J. Leprosy **4**:325, 1936.

PROTOZOAN DISEASES

Malaria.—Malaria from the standpoint of prevalence is no doubt the most important of all tropical diseases. It is one of the most frequent of all infectious diseases. It is well known throughout the world and is widely distributed over all parts of the tropical and subtropical regions. Records of the League of Nations from 65 countries showed 17,750,000 persons treated for this disease in 1932 alone (Brennan [176]). In India, with a population of 353,000,000, about 100,000,000 cases occur annually. Malaria is an infectious disease due to a parasite transmitted to man by the bite of an infected mosquito and characterized clinically by periodic attacks of fever associated with anemia. The causative parasite is a protozoan which passes its asexual cycle in man, who is the intermediate host, and its sexual cycle in the Anopheles mosquito, which is the definitive host. There are three types of this disease. The most common type is the benign tertian type, caused by Plasmodium vivax. This form is the most widespread and is the form seen most frequently throughout the temperate zones. The quartan malaria is relatively infrequent and is caused by Plasmodium malariae. The estivoautumnal or malignant tertain malaria is caused by Plasmodium falciparum and is encountered chiefly in the badly infected districts of the warmer parts of the world. It is the prevailing form in India, China and Central Africa and also is by far the most dangerous form because of its tendency to produce pernicious and malignant manisfestations, especially in regard to the central nervous system. In a recent bulletin of the United States Army Medical Department a series of 6,059 cases of malaria was reported in 140 of which, or 2.3 per cent, encephalitic malaria resulted (Fitz-Hugh and co-workers [177]).

The clinical picture in malaria affecting the brain is most variable, since almost any part of the organ may be involved, with concomitant symptoms. Often the clinical picture changes so rapidly that there is great overlapping of symptoms in a single case. It is for this reason that numerous types of cerebral malaria have been described, such as "meningeal type," "encephalic type," "cerebellar type," "myelitic type," "hemiplegic type" and so on. The onset of this disease varies within wide limits. Many of the patients are admitted to the hospital in coma, convulsions, delirium or merely lethargy; however, in most instances a careful history will reveal some premonitory symptoms during the preceding days consisting of intense headaches, nausea and vomiting, backache, nuchal pain, photophobia and vertigo. Generally the most common early complaints are intense headache, somnolence and disorientation.

176. Brennan, E. T.: M. J. Australia **1**:189, 1944.

177. Fitz-Hugh, T., Jr.; Pepper, D. S., and Hopkins, H. U.: Bull. U. S. Army M. Dept., 1944, no. 83, p. 39.

Much has been written on the lesions occurring within the brain in the so-called cerebral form of malaria. In the latter part of the nineteenth century Marchiafava and Bignami,[178] Mannaberg[179] and McCallum[180] described the cerebral changes fairly accurately from the point of view of the pathologists in some excellent monographs. Since that time considerable additions have been added to knowledge of the genesis and the pathologic aspects of malaria.

Probably the best known lesions are the vascular and hemorrhagic disturbances. For years the petechiae were considered as the classic lesions (Gaskell and Millar[181]; Dürck[182]; Rigdon[183]). These hemorrhages are of both the ball and the ring type and appear predominantly in the white matter. Dürck in a review of 30 cases of death from acute cerebral malaria was able to find that hemorrhages had occurred in but 40 per cent and felt that the associated cerebral changes were often more widespread and more important. Usually the capillaries and the smaller blood vessels show the most severe changes (Ogurtsova[184]; Dürck[185]; Margulis[186]; Gaskell and Millar[181]; Lafora[187]). Their endothelial cells are frequently swollen and even proliferated, reducing or even obliterating a part or the entire vascular lumen. Many of these cells are filled with pigment, fat granules or parasites. They often swell and break off into the lumen, producing a thrombus. Many of the cerebral capillaries are filled with malarial parasites, parasitized red cells or red cells filled with granules of pigment. The number of such thrombosed vessels varies from case to case, and in some instances they are extremely difficult to observe in spite of extensive clinical symptoms.

Probably more common than the vascular hemorrhagic lesions are the focal perivascular areas of necrosis, gliosis or both. Numerous variations have been described for these perivascular lesions, but probably all are related and represent variations of the same process (Marinesco[188]; Margulis[186]; Dürck[185]; Gaskell and Millar[181]; Dhayagude

178. Marchiafava, E., and Bignami, A.: On Summer-Autumn Malarial Fever, translated by J. H. Thompson, Publication 150, London, New Sydenham Society, 1894, p. 1.

179. Mannaberg, J.: The Malarial Parasites, translated by R. W. Felkin, Publication 150, London, New Sydenham Society, 1894, p. 235.

180. McCallum, W. G.: J. Exper. Med. **3**:103, 1898.

181. Gaskell, J. F., and Millar, W. L.: Quart. J. Med. **13**:381, 1920.

182. Dürck, H.: Arch. f. Schiffs- u. Tropen-Hyg. **29**:43, 1925.

183. Rigdon, R. H.: South. M. J. **37**:687, 1944.

184. Ogurtsova, A. S.: Nevropat. i psikhiat. **9**:42, 1940.

185. Dürck, H.: München. med. Wchnschr. **68**:33, 1921.

186. Margulis, M. S.: Neurol. Centralbl. **33**:1019, 1914.

187. Lafora, G. R.: J. f. Psychol. u. Neurol. **19**:209, 1912

188. Marinesco, M. G.: Brain **44**:223, 1921.

and Purandare [189]; Rigdon and Fletcher [190]). Margulis in 1914 first emphasized the presence of purely necrotic lesions in cerebral malaria. This type is invariably associated with a small occluded capillary containing pigment or parasitized red cells. The central necrosis reveals complete destruction of the brain tissue, which appears as an amorphous granular mass. Outward, toward the periphery of the lesion, there is less involvement, the tissues being fragmented and invaded by fat granule cells. These foci vary in number and in size, often measuring from 30 to 700 microns in diameter. Rigdon and Fletcher also described scattered areas of perivascular demyelination associated with these necrotic lesions.

When the purely necrotic type of lesion is surrounded by a peripheral zone of glial elements, it is usually called "Dürck's granuloma." In 1921 Dürck [185] emphasized the importance of this glial increase occurring particularly around the necrotic focus. He pointed out that the glial elements were frequently elongated and arranged radially about the necrotic focus. Red cells were occasionally observed around the glial elements. In the more chronic lesions the glial proliferation often extended inward to replace the necrotic tissue, resulting thus in a small patch of sclerosis.

Margulis [186] in 1914 was the first to describe glial nodules occurring in early malarial lesions entirely independent of necrosis of tissue. Similar lesions have been observed by Thomson and Annecke [191] and Marinesco.[188] In the cases of Thomson and Annecke these nodules were situated in the subcortical regions, while in Marinesco's case, they occurred chiefly in the gray matter and measured 210 to 130 microns in diameter.

A curious type of necrotic lesion was recently observed by Rigdon and Fletcher [190] in a case of cerebral malaria. They observed varying sized holes measuring 1.5 to 80 microns in diameter scattered throughout the white matter of the cerebrum and the cerebellum. Fragments of degenerating fibers were seen either free or extending across some of these spaces. These lesions they called "perforate lesions."

Damage of nerve cell in cerebral malaria was recognized as early as 1890 by Marchiafava and Bignami.[178] Lafora [187] in 1912 reviewed these neuronal alterations in detail. He described chromatolysis, swelling and vacuolation of the cytoplasm and degeneration of the medullated fibers. Since them, nerve cell changes have been observed by

189. Dhayagude, R. G., and Purandare, N. M.: Arch. Path. **36**:550, 1943.

190. Rigdon, R. H., and Fletcher, D. E.: Arch. Neurol. & Psychiat. **53**:191, 1945.

191. Thomson, J. G., and Annecke, S.: J. Trop. Med. **29**:343, 1926.

many investigators (Margulis [186]; Dürck [185]; Cerletti [192]; Rigdon and Fletcher [190]). The neuronal changes are most variable from case to case, often appearing in many scattered regions of the nervous system. Dürck [185] and Rigdon and Fletcher [190] described extensive degeneration and depletion of the Purkinje cells of the cerebellum. This localization of the changes to the cerebellum could well account for the occasional predominant cerebellar syndromes in this form of malaria. The nature of the nerve cell damage naturally varies with the severity and the chronicity of the involvement. Many of the cells reveal only mildly acute changes, while others reveal severe involvement, consisting of vacuolation, fragmentation and pyknosis. Invariably the damaged cells are observed scattered among intact elements, and in many areas the involved neurons predominate about the altered vessels. Dürck described extensive neuronophagia in some of his cases. Many of the damaged nerve cells evoked proliferation of glia, which ultimately invaded the damaged cells.

Actual inflammatory cellular reaction occurs infrequently in malaria. Gaskell and Millar [181] and Dürck [182] noted collections of lymphocytes around meningeal vessels.

A word might be said regarding present day views of the genesis of the cerebral lesions. By far the most popular and predominant view is that the cerebral lesions are vascular in origin and due to an embolic thrombotic process (Marchiafava and Bignami [178]; Dürck [182]; Dhayagude and Purandare [189]). Generally these investigators felt that the parasitized red cells, especially those with P. falciparum, had a tendency to agglutinate and adhere to the vessel wall. These agglutinated parasitized cells plus the swollen endothelial cells produced partial or complete vascular occlusion with resulting focal ischemic reactions. More recently various investigators, such as Gaskell and Millar [181] and Kean and Smith,[193] have questioned the thrombotic concept of these lesions. They were unable to find sufficient evidence of the so-called "cerebral plugging" to account for the severity of the cerebral symptoms. Mannaberg [179] as early as 1896 suggested that a toxin was liberated by the parasite which might account for the damage of the brain. The existence of such a toxin has been advocated by many investigators (Ewing [194]; Lafora [187]; Thomson and Annecke [191]). Most of these investigators based their opinions primarily on the fact that malarial coma occurs without parasites or pigment being found in the brain. However, the presence of such a toxin has never been demonstrated either in vivo or

192. Cerletti, U.: Histologische und histopathologische Arbeiten über die Grosshirnrinde, Jena, G. Fischer, 1910, vol. 4, p. 169.

193. Kean, B. H., and Smith, J. A.: Am. J. Trop. Med. **24**:317, 1944.

194. Ewing, J.: J. Exper. Med. **6**:119, 1902.

in vitro. More recently Rigdon [183] and Rigdon and Fletcher [190] have advocated the theory that anoxia is the basis for the cerebral lesions. These investigators argued that in severe malaria there is a rapid drop in hemoglobin and red cells resulting in severe anemia. The red cells surviving are often so heavily parasitized that their oxygen-carrying capacity is reduced. These two conditions, therefore, result in cerebral anoxia with the development of cerebral lesions, all of which can be reproduced in anoxic conditions of other etiology.

Trypanosomiasis.—The flagellate protozoa parasitic to man include those forms inhabiting the oral, intestinal and anal cavities, where they are but slightly pathogenic, and those living in the blood and the tissues, the hemoflagellates, which are highly pathogenic. Of the latter there are two groups, the trypanosomes, causing African and South American trypanosomiasis, and Leishmania forms, causing the various types of leishmaniasis. Both groups require invertebrate as well as vertebrate hosts to complete their life cycle, and both are more or less tropical in distribution.

(*a*) African Trypanosomiasis: African trypanosomiasis, or sleeping sickness, is caused by Trypanosoma gambiense, which is transmitted from man to man by the bite of species of tsetse flies. The infection is characterized by fever for a period, followed by an inflammatory reaction of the lymphatic system and by meningoencephalitis with symptoms of physical and mental lethargy. It occurs throughout equatorial Africa and is severe in some districts, being almost 100 per cent fatal if without treatment. Species of wild and domestic animals may act as a reservoir of the disease and in late years the incidence has been increasing in many areas.

Clinically the disease progresses from an early acute or incubation stage, lasting from a few months to several years, to a chronic cerebral or "sleeping sickness" stage with eventual fatal termination. The cerebral stage is characterized by the advent of various neurologic and psychiatric manifestations. Tremors, weakness, spasticity, and disturbances of personality become evident, and as the disease progresses the typical deterioration, emaciation and somnolence gradually increase until death intervenes. This stage begins early in the second year, occasionally sooner, and lasts from a few weeks to several months or even years. It is highly fatal. Death occurs from terminal meningitis, pneumonia or other secondary infections.

Although lesions of the central nervous system in sleeping sickness were described as early as 1840, the first adequate descriptions of the pathologic picture were that of Manson and Mott in 1900 [195] and that

195. Manson, P., and Mott, F. W.: Tr. Path. Soc. London **51**:99, 1900.

of Mott in 1906.[196] Mott characterized the disease as chronic adenitis followed by chronic inflammatory changes in the lymphatics of the brain and the spinal cord. These changes were manifested by proliferation and overgrowth of the neuroglia cells, especially of those related to the subarachnoid space and the perivascular lymph spaces. The leptomeninges contained in addition numerous lymphocytes. The perivascular neuroglial increase was most marked in the molecular layer of the cortex and in the subcortical white matter. Enclosed in the neuroglial network were lymphocytes, proliferated endothelial cells and plasma cells. The relative abundance of plasma cells in the perivascular spaces was characteristic. In the more chronic cases, a large type of cell with a deep blue nucleus and eosinophilic granules in the cytoplasm was observed. Cells of this type were called "morula cells" and were felt to be degenerated plasma cells. Capillary hemorrhages were found in all stages of the disease. Ganglion cells showed chromatolysis with little change in their size or shape. There was some diffuse atrophy of the fiber tracts in the white matter. Mott postulated that the clinical symptoms were caused by the altered function of ganglion cells, due to ischemia and improper nurition, and that the pathologic changes began with the entrance of trypanosomes into the cerebrospinal fluid. In a subsequent paper[197] he stressed the proliferation of endothelial cells and stated that the neuroglial composition of the perivascular infiltrate was much less extensive than he had previously indicated.

Similar observations were recorded by Warrington,[198] Low and Castellani[199] and Eisath.[200] Low and Castellani in addition found diffuse degenerative changes in the axis-cylinders and medullary sheaths in the spinal cord, and Eisath recorded glial cell enlargement and proliferation in the cord. Thomas and Breinl[201] reported a case in which capillary hemorrhages were extremely prominent, in places almost destroying the brain tissue. These petechiae were most marked in the pons and the spinal cord. Proliferation of the ependymal cells of the lateral ventricles was also seen. In another case there was considerable alteration in the large nerve cells of the brain and the cord with chroma-

196. Mott, F. W., in Reports of the Sleeping Sickness Commission, Royal Society of London, 1906, no. 7, vol. 15, p. 1.

197. Mott, F. W.: Proc. Roy. Soc. Med. **4**:1, 1910.

198. Warrington, W. B.: Brit. M. J. **2**:929, 1902.

199. Low, G. C., and Castellani, A., in Reports of the Sleeping Sickness Commission, Royal Society of London, 1903, no. 2, vol. 5, p. 14.

200. Eisath, G.: Arch. Path. **3**:647, 1927.

201. Thomas, H. W., and Breinl, A.: Trypanosomes, Trypanosomiasis, and Sleeping Sickness: Pathology and Treatment, Memoir 16, Liverpool School of Tropical Medicine, 1905.

tolysis, nuclear changes and ghost cells. Stevenson [202] demonstrated trypanosomes scattered throughout the brain substance, especially in the frontal lobes, the pons and the medulla, and more numerous in the white matter than in the cortex. In addition to the typical perivascular infiltration, he noted some vessels with thickened walls and contracted lumens but with few perivascular elements. He expressed the opinion that the perivascular infiltration developed when the trypanosomes migrated from the vessels to extravascular tissue. Peruzzi [203] found a relative increase in the number of "morula cells" in the infiltrations around vessels within the brain substance. They were also present in large numbers in the white and gray matter in close connection with nerve cells. He thought that they were of neuroglial origin and that their presence was accompanied by severe changes in nerve cells. He stressed the involvement of the choroid plexus and suggested that the trypanosomes gained access to the ventricular fluid through the choroid plexus. Spielmeyer [204] was of the opinion that the perivascular neuroglial proliferation was nonspecific and represented a reaction to inadequate cerebral nutrition resulting from the vasculitis. The cellular changes were most often seen in the deep layers of the gray matter with accompanying alterations of nerve fibers. He considered the "morula cells" to be degenerative and vascular, not neuroglial, in origin. Bertrand, Bablet and Sicé [205] after an extensive review of the literature and a study of untreated patients, concluded that from the very first sleeping sickness was a diffuse type of meningoencephalitis with marked diffuse perivascular infiltration. The cellular elements of the infiltration included both neuroglial and vascular elements with prominence of plasma cells. The white matter was usually involved to a greater degree, and the amount of neuroglial proliferation was related to some extent to the chronicity of the infection. Ganglion cell changes were slight and acute in nature. De Savitsch and Freeman [206] considered the principal effect of cerebral trypanosomiasis to be a diffuse change in the myelinated fibers with infiltration and isomorphic gliosis. They felt that the somnolence and the symptoms of deterioration resulted from interruption

202. Stevenson, A. C.: Proc. Roy. Soc. Trop. Med. & Hyg. **16**:135 and 384, 1922.

203. Peruzzi, M.: Pathologico-Anatomical and Serological Observations on the Trypanosomiases, Final Report of the League of Nations International Commission on Human Trypanosomiasis, Geneva, Switzerland, 1927, vol. 3.

204. Spielmeyer, W.: Die Trypanosomenkrankheiten und ihre Beziehungen zu den syphilogenen Nervenkrankheiten, Jena, G. Fischer, 1908.

205. Bertrand, I.; Bablet, J., and Sicé, A.: Ann. Inst. Pasteur **54**:91, 1935.

206. de Savitsch, E., and Freeman, W.: M. Ann. District of Columbia **8**:231, 1939.

of the association pathways. Only in the floor of the third ventricle were the infiltrations so extreme as to invade the neighboring cerebral tissue.

(*b*) South American Trypanosomiasis: South American trypanosomiasis, or Chagas' disease, was first described by Chagas [207] in Brazil in 1909. It is caused by the flagellate Trypanosoma cruzi, which is transmitted from the animal reservoir to man by a species of reduviid bugs. Acute and chronic forms of the disease are described. In the acute stage the parasites are found in the circulating blood and produce fever, edema, adenitis and convulsions, while in the chronic forms the symptoms are produced by the parasites as they become localized in various tissues of the body, especially the heart and the brain. The cases of this disease are scattered throughout South and Central America, but most of the reported cases occurred in Brazil and Argentina. It is not a common infection, and the cases are numbered only in the hundreds. Most of the patients have been infants and young children, and it is among this age group that the acute form with a high mortality rate occurs. Adults infected experimentally have suffered only mild symptoms, and in some the disease has been discovered only by routine examination of the blood.

Symptoms of involvement of the nervous system are particularly evident during the acute phase, and in many cases comprise the greater part of the clinical picture. Convulsions are most common and are usually present terminally, though they may occur initially or at any time during the disease. Many symptoms referable to the nervous system, such as mental retardation, apathy, speech disorders and idiocy, are described in chronic cases, but are most likely due to cretinism rather than to trypanosomiasis, though sequelae from severe infections might be quite similar. Paralysis, disorders of locomotion, incoordination, convulsions and coma have been noted in dogs experimentally infected.

The general pathology of Chagas' disease has been summarized by Yorke [208] as indicating degeneration of the invaded cells with accompanying cellular infiltration and eventual fibrosis of the affected tissues. Heart, brain and liver are most severely involved. Inside the cell the parasite has a form resembling Leishmania, being a small round or oval body with a nucleus and a kinetoplast. In this form it multiplies within the cell, forming intracellular cysts, which eventually rupture, liberating the parasites into the surrounding tissue.

The involvement of the nervous system was first described completely from the point of view of the pathologist by Vianna.[209] He found inflam-

207. Chagas, C.: Mem. Inst. Oswaldo Cruz **8**:5, 1916.
208. Yorke, W.: Trop. Dis. Bull. **34**:275, 1937.
209. Vianna, C.: Mem. Inst. Oswaldo Cruz **3**:276, 1911.

matory nodules in the gray and the white matter in all parts of the central nervous system, but most numerous in the basal nuclei, the pons and the spinal cord. They were of varying size and had no direct relationship to the neighboring vessels. They were frequently surrounded by a diffuse zone of leukocytic infiltration. The parasites were seen inside round mononuclear cells which, though difficult to recognize because of distortion, were thought to be neuroglia cells. Inflammatory changes were present in the meninges, and there was slight perivascular infiltration. Torres and Villaca [210] confirmed these observations and stated that the cells making up the inflammatory nodules were neuroglia and mononuclear cells.

Crowell [211] in studying an 8 month old infant who died during an acute infection found similar changes in the brain. The parasitized cells were few and were not seen near inflammatory foci such as those just described. De Coursey [212] found in addition to the inflammatory foci mild perivascular infiltration with round cells and occasional polymorphonuclear cells. The foci were composed of irregularly flame-shaped nests of small round cells with little cytoplasm. There were infrequent large cells whose distended cytoplasm contained 20 to 80 leishmaniform parasites. Lundeberg [213] described foci of microglia cells with round, oval or vesicular nuclei and pale cytoplasm. Some of the cells contained clusters of parasites, and in some places the parasites appeared to lie free in nerve fibers. Many small monocytes were scattered among the larger parasitized cells. The foci were frequently associated with small blood vessels, the latter usually exhibiting marked swelling and hyperplasia of the endothelium. A few degenerated nerve cells and an occasional capillary hemorrhage were noted.

Johnson and de Rivas [214] examined a 3 month old infant in whom the meninges were so edematous as to present the appearance of a gelatinous capsule covering the brain. A small amount of clear fluid expressed contained motile trypanosomes. Inflammatory foci of mesoglial cells and monocytes, some with areas of necrosis, were scattered throughout the nervous system, but parasites were found only in the cerebrum. Parasites were seen within the protoplasmic astrocytes, also, but no inflammatory reaction was observed around these cells.

Mazza and his associates [215] concluded that pathologic study provided no characteristic cytologic picture which would make one think

210. Torres, C. M., and Villaca, J.: Mem. Inst. Oswaldo Cruz **11**:80, 1919.
211. Crowell, B. C.: Am. J. Trop. Med. **3**:425, 1923.
212. de Coursey, E.: Am. J. Trop. Med. **15**:33, 1935.
213. Lundeberg, K. R.: Am. J. Trop. Med. **18**:185, 1938.
214. Johnson, G. M., and de Rivas, G. T.: Am. J. Trop. Med. **16**:47, 1936.
215. Mazza, S.; Friere, R. S., and Salica, P. N.: Investigaciones sobre la enfermedad de Chagas, Regional Argentina, University Buenos Aires, 1942.

of the disease. They found the meninges extensively and diffusely infiltrated by leukocytes which advanced along the perivascular spaces deep into the white substance of the brain. The infiltration extended outward into the circumscribed nodules, but its perivascular nature was still evident. The cellular elements consisted of macrophages, polymorphonuclear leukocytes and mononuclear cells. In addition proliferation of the glial elements occurred, and in the more severely involved areas degeneration of brain tissue. The distribution of parasites was roughly parallel to the intensity of the leukocytic reaction. Organisms were occasionally seen in glial cells, fat granule cells and macrophages or free from cells in nerve tissue or the perivascular space.

Most of the changes described by the foregoing authors were demonstrated experimentally in dogs by Villela and Torres.[216] Gross examinations yielded negative results. Microscopically the most prominent lesions were small inflammatory foci seen in the neighborhood of precapillary or capillary vessels in both the gray and the white matter in all parts of the central nervous system, though less often in the cerebellum. The structure of these foci varied with their age. In the first stages they were made up of macrophages, leukocytes and some plasma cells, generally in close relationship to the vessels. In the more advanced foci, the fat granule cells were more prominent, and there was evident disintegration of nerve tissue with formation of fissures and cavities. In the oldest foci, macrophages were no longer seen, and the cells present had become elongated to resemble fibrous neuroglia. The vascular lesions consisted of perivascular infiltrations of endothelial cells, lymphocytes and plasma cells. These cells first lodged in the adventitia and thence spread to the lymphatic spaces in the more intense reactions. The endothelial cells were always dominant; they rarely contained parasites. The vascular infiltration was never diffuse but limited to a single vessel or part of a vessel. The most commonly involved nerve cells were the Purkinje cells of the cerebellum and the pyramidal cells of the cerebral cortex. The lesions of these cells consisted of swelling and vacuolation or of shrinking and atrophy. Neuronophagia was common. Parasites were observed most often in the protoplasm of macrophages, in the fat granule cells and occasionally in the protoplasmic neuroglia. It appeared that the parasites pierced the capillary wall and were taken up by the neuroglial cells. The infected cells then became foci for the infection of other cells.

(*c*) Leishmaniasis: Leishmaniasis is caused by a protozoan parasite belonging to the genus Leishmania. It is made up of two forms, a visceral

216. Villela, E., and Torres, C. M.: Mem. Inst. Oswaldo Cruz **19**:198, 1926.

form commonly known as kala-azar and a cutaneous form known as tropical or oriental sore.

Kala-azar is an infectious disease caused by Leishmania donovani and probably spread by the sandfly. Although a tropical disease, it does not occur in the hot climates and is more prevalent in the cooler seasons. It is found in Africa, the Mediterranean area of Europe, and Asia, being extremely frequent in India, China and Manchuria. The parasite as seen in man is known as the Leishman-Donovan body. It is a round or oval unicellular organism measuring 2 to 5 microns in diameter and is found chiefly in the reticuloendothelial cell or the tissue macrophage. It multiplies within the cell of the host, replacing the entire cell, which ultimately becomes destroyed, liberating the parasites. Experimentally dogs, cats, mice, hamsters and monkeys can be infected with this disease. The dog may be the principal animal reservoir of the disease in certain localities.

The incubation period of kala-azar is from two to four months. The onset may be sudden or gradual, being ushered in by such symptoms as fever, chills, vertigo, headache, malaise, abdominal pain, nausea and vomiting. The temperature curve is irregular. There is progressive loss of weight with general wasting. Most characteristic of this disease is the enlargement of the spleen and the liver. Involvement of the nervous system is most unusual. The headaches are never severe, and mental symptoms rarely occur even when the fever is high.

In view of the infrequency with which this disease involves the central nervous system, reports on the pathologic changes within the brain are few and based mostly on study of animal tissues.

La Cava [217] in 1911 published studies of a fatal case of infantile leishmaniasis. The dura was covered with petechiae and the meninges were congested, but the brain seemed uninvolved. Similar findings have been reported by Christophers.[218]

Microscopically, parenchymatous lesions have been reputed not to occur in the brain of man. In experimental animals, parasites have not been found in the brain but have been found in the meninges. Meleney [219] studied material from 19 hamsters inoculated with L. donovani. Parasitized cells were observed only in the meninges and the choroid plexus, usually in the loose tissue just outside the blood vessels. Nicolau and

217. LaCava, F.: Atti Roy. Acad. Lincei R. C. **20**:778, 1911.

218. Christophers, S. R.: On a Parasite Found in Persons Suffering from Enlargement of the Spleen in India, Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India, Calcutta, Supt. Gov. Print., 1904, no. 11.

219. Meleney, H. E.: Am. J. Path. **1**:147, 1925.

Perard [220] observed similar localizations of canine leishmaniasis. They surmised that the choroid plexus arrested the organisms and prevented them from reaching the brain. In only 1 dog did they find an intracerebral lesion; this was in the region of the floor of the fourth ventricle. There was an intense parenchymatous reaction with infiltrations of mononuclears, polymorphonuclears and plasma cells. An occasional degenerated parasite could be seen in the cellular reaction. Hoeppli [221] observed some neuronophagia and degeneration of ganglion cells in hamsters experimentally infected with L. donovani and L. tropica. Even when a suspension of Leishman-Donovan bodies was inoculated intracerebrally in Chinese hamsters, focal parenchymatous lesions failed to develop.

Relapsing Fever.—Relapsing fever is a febrile spirochetal infection which is widely distributed in many parts of the world and is transmitted to man by the tick and the body louse. There are many different species of spirochetes producing relapsing fever. Some of the more common species are: S. recurrentis (European), S. duttoni (Central African), S. novyi (American), S. berbera (North African). In general the European, Indian, Chinese and West African infections are transmitted from man to man by body lice, while the Central African and American forms are transmitted by ticks. In Africa, relapsing fever ranks next to malaria in frequency and is found chiefly in Egypt, Algeria and West Africa. Relapsing fever has appeared recently in endemic foci in many states within the United States. From Texas alone 258 cases occurring between 1930 and 1935 were reported by Kemp and his associates,[222] while 138 cases were studied in California between 1930 and 1938 by Wheeler.[223]

Clinically there is an acute onset of fever, malaise and headaches. The temperature rises rapidly and remains elevated for a number of days, falling by crisis. After an afebrile period of four to eight days there is a similar but usually milder febrile episode. In the European type there are usually two to three paroxysms, while in the African type there may be as many as ten relapses. Neurologic complications are most frequent in the tick-borne infections and usually occur late. The frequency of cerebral involvement is difficult to compute and has varied from 4.5 per cent of cases (Adler and co-workers [224]) to 20 per cent (Cooper [225];

220. Nicolau, S., and Perard, C.: Ann. Inst. Pasteur **57**:463, 1936.

221. Hoeppli, R.: Arch. f. Schiffs- u. Tropen-Hyg. **33**:101, 1929.

222. Kemp, H. A.; Moursund, W. H., and Wright, H. E.: Am. J. Trop. Med. **13**:425, 1933.

223. Wheeler, C. M.: Am. J. Trop. Med. **18**:431, 641, 1938.

224. Adler, S.; Theodor, V., and Schrieber, H.: Ann. Trop. Med. **31**:25, 1937.

225. Cooper, E. L.: M. J. Australia **1**:635, 1942.

Scott [226]). Experimentally it has been recognized for years that the spirochete of relapsing fever is specifically neurotropic (Velu and co-workers [227]; Mathis and Durieux [228]; Levaditi and Anderson [229]; Lagrange [230]). Heronimus [231] thought that the brain was probably always infected in this disease. Plant [232] showed that the nervous system of the rabbit, which has natural immunity to spirochetal infection, does not have such immunity to infection with S. duttoni and can be infected while other organs remain free.

Clinically the most common neurologic complaint is intense headache, often occipital in distribution. The headache recurs with each attack of pyrexia and often persists for weeks after the temperature subsides (Cooper [225]). The headache is usually accompanied by vertigo, insomnia and, in severe forms, by delirium. Probably the most frequent involvement in relapsing fever is that of the meninges (Ligeois and co-workers [233]; Lafforgue [234]; Scott [226]; Cooper [225]; Stones [235]). Cooper reported that one fifth of his patients had meningeal symptoms. Symptoms of encephalitic involvement have also been reported (Vialatte [236]; Liegeois and co-workers [233]; Scott [226]).

Pathologic changes within the nervous system in relapsing fever are not too numerous in spite of the neurotropism of the organism. Belezky and Umanskaja [237] reported the most complete study of the encephalic changes in man, based on 8 fatal cases. The leptomeninges were hyperemic, and the vessels were surrounded by monocytes, lymphocytes, plasma cells and a few red cells. Spirochetes were observed free in the subarachnoid space as well as within the pial veins. The cerebral cortex showed extensive degeneration of ganglion cells. Many of the neurons were acutely swollen and showed complete chromatolysis and some vacuolation. There was mild glial increase. Degenerated spirochetes were present throughout the second and the fourth cortical layers,

226. Scott, R. B.: Lancet **2**:436, 1944.

227. Velu, H.; Balozet, L., and Zoltner, G.: Compt. rend. Soc. de biol. **106**: 1089, 1931.

228. Mathis, C., and Durieux, C.: Bull. Soc. path. exot. **23**:862, 1930.

229. Levaditi, C., and Anderson, T. E.: Compt. rend. Soc. de biol. **100**:1121, 1929.

230. Lagrange, E.: Bull. Soc. path. exot. **24**:804, 1931.

231. Heronimus, E. S.: Zentralbl. f. Bakt. (Abt. 1) **105**:394, 1928.

232. Plant, F.: München. med. Wchnschr. **73**:1552, 1926.

233. Liegeois, R.; Pages, R.; Duguet, J., and Pouhin: Presse méd. **46**:531, 1938.

234. Lafforgue: Rev. de méd., Paris **28**:916, 1908.

235. Stones, R. Y.: Kenya M. J. **3**:27, 1926.

236. Vialatte, C.: Arch. Inst. Pasteur d'Algérie **4**:56, 1926.

237. Belezky, W. K., and Umanskaja, R. M.: Ztschr. f. d. ges. Neurol. u. Psychiat. **129**:21, 1930.

unrelated to the vessels. Some spirochetes were found in the glial cells but none in the white matter. The basal ganglions showed acute swelling of the cells. Spirochetes were present in the floor of the fourth ventricle between the ganglion cells of the olive. Jahnel and Lucksch [238] made sections of the brains of 2 soldiers who died of relapsing fever and found spirochetes in both. These were observed chiefly in the vessels, although a few were lying free between the cortical neurons. There were some petechiae in the pia.

Levaditi and co-workers [239] described in detail the changes in the brains of rabbits and monkeys infected with relapsing fever. These resembled closely the changes observed in man. The meninges contained many perivascular and diffuse accumulations of monocytes. The most severe encephalic changes occurred in the cortex, in the white matter along the walls of the lateral ventricles, and within the midbrain and the medulla. The changes were chiefly parenchymatous and vascular. The former consisted of foci of mononuclears surrounding many vessels and extending outward to involve the neurons and the neuroglial elements. The perivascular accumulations were tremendous, often being ten to twenty layers deep. The vessels frequently showed endothelial increase with narrowing of the lumen. Many of the nerve cells showed chromatolysis and vacuolation.

Rat Bite Fever.—Rat bite fever is an acute infectious disease caused by Spirochaeta morsus muris, transmitted usually in the bite of a rat, less commonly in the bite of a ferret, a cat, a weasel, a dog or a squirrel (Ripley and Van Sant [240]). The disease is characterized by paroxysms of fever, an inflammatory reaction at the site of the bite, tenderness in the regional glands, an exanthem and various, often marked neurologic symptoms.

This disease has been recognized in India for many centuries and is very common in the Orient. It is endemic in Japan, where the mortality rate in untreated patients is 10 per cent. Within the last twenty years cases have been reported from Spain, Italy, France, Germany, Australia and America. According to Brown and Nunemaker,[241] 125 cases proved to be cases of rat bite fever were reported in the United States through 1940. In these there were no deaths.

In 1914 Schottmüller [242] described a case of rat bite fever from which a "streptothrix" was isolated. Similar organisms have been

238. Jahnel, F., and Lucksch, F.: Med. Klin. **23**:2003, 1927.

239. Levaditi, C.; Anderson, T. E.; Selbie, F. R., and Schoen, R.: Bull. Acad. de méd., Paris **103**:673, 1930.

240. Ripley, H. S., and Van Sant, H. M.: J. A. M. A. **102**:1917, 1934.

241. Brown, T., and Nunemaker, J. C.: Bull. Johns Hopkins Hosp. **70**:201, 1942.

242. Schottmüller, H.: Dermat. Wchnschr. **58**:77, 1914.

isolated from other cases of rat bite fever and the type has been called Streptobacillus multiformes (Lemierre and associates [243]; Place, Sutton and Willner [244]). The rat bite fever caused by this type of organism is clinically more benign and when compared with the form generally accepted in the foreign literature as caused by Spirillum minus (Spirochaeta morsus muris) is found somewhat dissimilar. In this study the true spirillum disease is referred to.

For a short time rat bite fever, because of its relapsing nature and the complete arsenical control of it, was used for the treatment of the meningoencephalitic type of psychosis with syphilis of the central nervous system (general paresis). Solomon and co-workers [245] instituted this treatment in 1926 when he inoculated 12 patients suffering from this condition. Since then 104 cases of rat bite fever have been artificially produced by inoculation of Spirochaeta morsus muris (Bayne-Jones [246]; Hershfield and co-workers [247]; Teitelbaum [248]). However, this form of treatment has been abandoned because of the 10 per cent mortality with some strains of this organism and because of the high incidence of complications (arthritis, myositis, convulsions and others).

Neuropsychiatric symptoms comprise some of the most common complaints in this illness and invariably appear at the height of the first few paroxysms of temperature. Patients with the most severe form complain of headache, vertigo, tinnitis, nausea and vomiting, and blurring of vision (Arkin [249]; Gilkey and Dennie [250]; McDermott [251]). Generally these symptoms subside with the fall of temperature. However, in some of the more severe infections the neurologic involvement is more extensive and more lasting. There may be lesions of cranial nerves, producing dysphagia, aphonia, amaurosis, deafness and papilledema (Ebert and Hesse [252]; Rasdolsky [253]). Motor disturbances are often quite definite and in certain patients may persist beyond the paroxysm as a permanent sequel. These motor symptoms consist of muscular twitchings, convul-

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243. Lemierre, A.; Reilly, J.; LaPorte, A., and Morin, M.: Bull. Acad. de méd., Paris **117**:705, 1937.

244. Place, E. H.; Sutton, L. E., and Willner, O.: Boston M. & S. J. **194**:285, 1926.

245. Solomon, H. C.; Berk, A.; Theiler, M., and Clay, C. L.: Arch. Int. Med. **38**:391, 1926.

246. Bayne-Jones, S.: Internat. Clin. **3**:235, 1931.

247. Hershfield, A. S.; Kibler, O. A.; Colby, S.; Koenig, M. T.; Schmid, O. W., and Saunders, A. M.: J. A. M. A. **92**:772, 1929.

248. Teitelbaum, A. D.: M. Bull. Vet. Admin. **6**:263, 1930.

249. Arkin, A.: Arch. Int. Med. **25**:94, 1920.

250. Gilkey, H. M., and Dennie, C. C.: South. M. J. **32**:1109, 1939.

251. McDermott, E. N.: Quart. J. Med. **21**:433, 1928.

252. Ebert, B., and Hesse, E.: Arch. f. klin. Chir. **136**:69, 1925.

253. Rasdolsky, I.: Ztschr. f. d. ges. Neurol. u. Psychiat. **90**:188, 1924.

sions, hemiplegia, paresis and even muscular atrophies. Sensory involvement usually occurs in the most severe type of the disease and varies from paresthesias to areas of complete anesthesia, particularly in the extremities.

In spite of the fact that cerebral involvement seems to be very frequent in this disease, reports of autopsy studies on the central nervous system are greatly lacking in the literature. Miyaki [254] noted increase of spinal fluid and congestion of the vessels of the meninges and the cord. No histologic studies have been reported. Kaneko and Okuda [255] reported autopsy studies on a 70 year old man dying from rat bite fever. The age of this patient makes the histologic observations of questionable significance. The brain appeared hyperemic. Microscopically the neurons within both the brain and the cord showed definite swelling and fragmentation. Scattered cells were pyknotic. The nerve fibers revealed slight swelling and disintegration. Dragisic and Kaludjerski [256] reported a fatal case of rat bite fever. The patient, a 14 month old child, presented unilateral convulsive seizures. A spirillum was isolated from the blood. Grossly, a yellowish softened area filled with petechiae was observed in the genu of the corpus callosum. The cerebral tissues were hyperemic. Microscopically the involved area of the brain revealed numerous small foci of glia cells and perivascular erythrocytes. The softened area within the corpus callosum was filled with necrotic tissue and blood.

Amebic Dysentery.—Amebiasis is considered and classified as a tropical disease even though it occurs throughout the world and is extremely common in the temperate zones. The general idea prevails that "amebic dysentery" and "amebiasis" are synonymous terms and that amebic infections are uncommon except in the tropics. According to Craig,[257] 5 to 10 per cent of all the inhabitants of the United States are infected with Endamoeba histolytica.

E. histolytica primarily infects the intestine, reaching the bowel in drinking water or food which have been contaminated with fecal material containing amebic cysts. From the intestinal lesions the amebas may metastasize through the portal vein to the liver and rarely to other organs, such as the brain, where they set up a primary purulent encephalitis or an abscess of the brain. It has been estimated that hepatic abscesses occur in from 33 to 51 per cent of persons with amebic dysentery coming to autopsy, while only 3 per cent of these show

254. Miyaki, H.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **5**:231, 1899.
255. Kaneko, R., and Okuda, J.: J. Exper. Med. **26**:363, 1917.
256. Dragisic, B., and Kaludjerski, S.: Arch. f. Kinderh. **112**:168, 1937.
257. Craig, C. F.: J. A. M. A. **77**:827, 1921.

associated involvement of the brain (Clark [258]; Gehlen [259]; Kartulis [260]). About 65 cases of amebic abscess of the brain have been reported in the literature. In most of the cases there was also an associated abscess of the lung or the liver. In only 5 cases was the involvement of the brain an isolated finding (Kartulis [260]; Putney and Baker [261]; Stein and Kazan [262]).

When the brain is invaded by the endameba, there occur the symptoms and signs of diffuse suppurative meningo-encephalitis with the focal signs of an abscess. Generally the cerebral symptoms are preceded by signs of hepatic or thoracic involvement, often following operations for such complications.

The histologic changes occurring in amebic infections of the brain will naturally vary with the nature and the age of the infective process (Halpert and Ashley [263]; Kartulis [260]; Stein and Kazan [262]). Early lesions will reveal vascular congestion and thrombosis with secondary softening of tissue. Leukocytes, red cells and many amebas fill the softened area, which disintegrates to form a cavity filled with necrotic debris and surrounded by a rough, shaggy wall. In the smaller focal lesion this cavity becomes surrounded by a thick capsule composed of connective tissue, glial elements, blood vessels and mononuclear cells. The inner wall of the capsule often is composed of fat granule cells which have phagocytosed much of the necrotic brain tissue. Amebas often can be found in the abscess wall, appearing as hyaline masses or resembling compound granular cells, although they seem to be larger and possess a condensation of the outer cytoplasmic wall giving the cell a ringlike appearance. According to Stein and Kazan,[262] the amebas stain best with Best's glycogen stain, although they can be seen clearly with hematoxylin-eosin stain. The thickness of the abscess wall depends on the chronicity of the process. The small lesion may be practically replaced by the proliferative changes, while the larger one reveals a large purulent center filled with yellowish-brownish gelatinous pus and usually well walled off by a heavy capsule.

In the more extensive lesions, large areas of brain tissue become implicated, so that extensive softening occurs, surrounded by a wide zone of cerebral edema (Stein and Kazan [262]). This entire involved region reveals rarefied brain tissue filled with atrophic cellular elements and invaded by collections of mononuclear cells, fat granule cells and

258. Clark, H. C.: Am. J. Trop. Med. **5**:157, 1925.
259. Gehlen, J. N.: Minnesota Med. **17**:18, 1934.
260. Kartulis: Zentralbl. f. Bakt. (Abt. 1) **37**:527, 1904.
261. Putney, F. J., and Baker, D. C.: Dis. of Chest **4**:20, 1938.
262. Stein, A., and Kazan, A.: J. Neuropath. & Exper. Neurol. **1**:32, 1942.
263. Halpert, B., and Ashley, J. D.: Arch. Path. **38**:112, 1944.

extravasated red cells. Many amebas can be found within the softened brain tissue. Scattered in this area of more diffuse involvement are often seen smaller foci of actual necrosis of tissue with liquefaction and true abscess formation, so that numerous tiny abscesses can be identified throughout the involved region. Many of these small abscesses are surrounded by capsules of varying thickness. The small abscess is composed of a necrotic center containing leukocytes and red cells in varying stages of disintegration. Around the disintegrated center is a layer of fat granule cells, surrounded in turn by a firm layer of granulation tissue. Amebas are observed chiefly in the less severely involved tissue; that is, they are in the abscess walls, in the foci of rarefaction of tissue or within the meninges.

The meninges when implicated are thickened, containing aggregates of lymphocytes, polyblasts and fibroblasts.

Alexander and Wu [264] in studying a case of chronic amebic dysentery reported nonspecific and nonsuppurative changes resembling those seen in bacillary dysentery. They observed ischemic foci throughout the cerebral cortex, often replaced by glial scars. There were lipoid deposits in many of the cortical neurons. In the cerebellar cortex some of the Purkinje cells had disappeared and were replaced by glial shrubs. With silver stains the nerve cells revealed crumbling and destruction of the intracellular neurofibrils associated with argentophilia of the nuclei and of some of the glial cells.

ANIMAL PARASITES (HELMINTHS)

Ascariasis.—Ascariasis is caused by infection with the roundworm Ascaris lumbricoides, the helminth most commonly parasitic in man. It is worldwide in its distribution but is more prevalent and severe in the tropics owing to environmental conditions and to insanitary practices.

A number of cases are recorded in which symptoms suggesting meningeal and cerebral involvement have been ascribed to the toxic effects of the worms. Kollman [265] reviewed the literature and recorded cases in which meningitis, epileptiform convulsions, choreiform movements with delirium and paralysis of the lower extremities were symptoms which cleared up immediately on passage of the ascarids. Langhans [266] and Steber [267] described cases in which marked restlessness and delirium cleared up with removal of the worms. Cases simulating those of

264. Alexander and Wu, footnotes 109 and 112.

265. Kollman, A.: Arch. f. Kinderh. **82**:150, 1927.

266. Langhans, G. L.: Ztschr. f. Kinderh. **39**:344, 1925.

267. Steber: Deutsche med. Wchnschr. **43**:1040, 1917.

meningitis have been described by Turcan,[268] Valerio,[269] Ferrari [270] and Spieth.[271]

It is not clear whether these symptoms are produced by toxins elaborated by the living worms or by toxins released after their death in the intestine. Rachmannow [272] injected extracts of the worms into guinea pigs and found lesions of the central nervous system only in those animals which displayed clinical signs of involvement of the nervous system. The lesions he described were various degrees of chromatolysis of ganglion cells, nuclear changes with pyknosis and destruction of neurofibrillae. Neuroglial changes consisted of the appearance of ameboid cells and satellitosis. In addition there were patchy areas of demyelination in the white matter.

Cerebral symptoms may occasionally result when the larvae accidentally reach the general circulation and the brain. Fülleborn [273] found larvae in the brain a few days after feeding eggs of Ascaris to experimental animals, but he never noted cerebral symptoms even if the larvae were injected into the carotid artery. He surmised that most of the larvae passed through the brain back to the lungs and that hemorrhages occurred only rarely in the relatively firm brain tissue. Hoeppli [274] after feeding eggs to guinea pigs found larvae in the ventricle, starting to bore into the brain tissue. There was no surrounding reaction. In an animal killed six weeks after it had ingested eggs, he found a fully grown larva in a capillary in the brain stem, with no surrounding tissue changes. Yamaguchi [275] found larvae in the brain substance, less frequently in small arteries, and in the lateral ventricle and the choroid plexus in his animals. Occasionally he saw the moulted skins of larvae in the brain tissue. Meningeal and perivascular hemorrhages were fairly frequent, but little bleeding was noted within the brain tissue itself. The hemorrhages were most abundant in the cerebellum and the floor of the fourth ventricle. He also found a few nodules of proliferative glial cells scattered throughout the brain.

Hookworm Infection.—Hookworm infection (ancylostomiasis) is most prevalent in the tropical and subtropical countries of the world. The two most common species of worms causing the disease are Necator americanus, found in the New World and in equatorial Africa, and Ancylostoma duodenale, found in the Mediterranean regions and Asia

268. Turcan, H.: Presse méd. **30**:844, 1922.
269. Valerio, A.: Brasil-med. **40**:309, 1926.
270. Ferrari, G.: Gior. di clin. med. **14**:745, 1933.
271. Spieth, H.: Virchows Arch. f. path. Anat. **215**:117, 1914.
272. Rachmannow, A.: Ann. Inst. Pasteur **28**:181, 1914.
273. Fülleborn, F.: J. Helminthol. **7**:15, 1929.
274. Hoeppli, R.: Virchows Arch. f. path. Anat. **244**:159, 1923.
275. Yamaguchi, S.: Arch. f. Schiffs- u. Tropen-Hyg. **29**:589, 1925.

Minor. Both species are prevalent throughout the Orient. An infection rate of over 50 per cent prevails in most areas.

The symptoms referable to the nervous system are prominent. Sandwith [276] in 1894 stressed the symptoms of lethargy, eating dirt (geophagia), impotence, stupidity and decrease of the patellar reflexes. Ashford and King [277] noted headache, lack of attention to details and slight dulling of the mental faculties in patients with mild infections. In those whose disease was moderate the appetite was more exaggerated and dizziness, frequent headache, tingling of the feet, diminution of the patellar reflexes, passivity and mental depression were found. In those with severe disease there were dizziness, tinnitus, insomnia, absence of the patellar reflexes, impotence and amenorrhea, dulling of the intellect, depression, morbid parasthesias, blurred vision and extreme weakness.

The only suggestion that any pathologic changes take place in the nervous system with hookworm disease has been provided by Fülleborn.[278] He injected larvae into the jugular vein of the experimental animal and succeeded in recovering larvae from the brain. In an animal killed twenty-five minutes after the injection, two living larvae were found in the brain; in a second animal killed six and a half hours after the injection, five larvae were recovered and a small hemorrhage was seen in the brain tissue. In view of the path which the migrating larvae follow, it is possible that some larvae might reach the brain through the general circulation and cause symptoms.

Strongyloidiasis:—Strongyloides stercoralis has a life history somewhat similar to that of hookworm and produces a similar pathologic picture. It is capable of living outside the body indefinitely, and autoinfection may take place. The chief symptoms, according to Hinman,[279] are vague abdominal pains, diarrhea sometimes alternating with constipation, and loss of weight. The variability of the symptom complex is striking.

Faust [280] made a pathologic study of the infection in a series of animals. Four of 62 dogs were killed between eight and twenty days after exposure, because of symptoms indicating damage of the nervous system. One of these animals was in a state of tetany with spasticity of the left extremities and of the right side of the face. Incontinence subsequently developed. Similar symptoms were found in 2 additional dogs, and in the fourth dog a syndrome suggesting rabies developed on the eighth day. Microscopic examination of brain tissue showed scattered capillary hemorrhages, but no larvae were found. Yama-

276. Sandwith, F. M.: Lancet **1**:1362, 1894.
277. Ashford, B. K., and King, W. W.: J. A. M. A. **49**:471, 1907.
278. Fülleborn, F.: Arch. f. Schiffs- u. Tropen-Hyg. **30**:679, 1926.
279. Hinman, E. H.: Rev. Gastroenterol. **5**:24, 1938.
280. Faust, E. C.: Arch. Path. **19**:769, 1935.

guchi [275] reported more severe but similar findings. Hemorrhages were numerous in the meningeal and the perivascular tissues, and they were more severe in the cerebellum. The areas most involved in the cerebrum were the cortex and the white matter, and especially the floor of the fourth ventricle. Larvae were seen free in the brain, in the small vessels and in the ventricles and the choroid plexus. A few proliferative glial nodules were seen scattered throughout the brain.

Filariasis.—The filarial worms are slender nematodes parasitic in the lymphatic and circulatory systems and the deeper tissues of the body. The adult females produce young prelarval worms known as microfilarias, which circulate in the blood and the lymph stream of the host. The life cycle is completed in an arthropod vector, and the worms are transmitted from man to man by various species of mosquitoes and biting flies.

Infection with Wuchereria bancrofti, commonly called filariasis, is the most widespread of all the filarial infections. In this disease the adult worms are found chiefly in the lymphatics. The incubation period is about a year, and the infection may remain entirely asymptomatic or may be evidenced by recurring attacks of lymphangitis. The chronic stages occur through lymphatic obstruction, with dilated lymphatics, enlarged lymph glands, lymphoceles, hydrocele and elephantiasis resulting. The circulating microfilarias apparently produce no symptoms.

Several investigators have thought, however, that the symptoms of involvement of the nervous system occasionally seen in filariasis are due to the presence of microfilarias in the brain. Mya [281] described the case of a patient admitted to the hospital for sudden loss of consciousness four hours previously. The next day the patient was semiconscious. Right hemiplegia developed and the patient died sometime later. The only positive finding was the presence of microfilarias in the spinal fluid. Manson [282] stated that microfilarias can be found in the brain, and Anderson [283] reported a case in which numerous microfilarias were observed in the cerebral and cerebellar blood vessels. Some leukocytic infiltration was present around cerebral capillaries. Rodenwaldt [284] also found microfilarias in the cerebral capillaries, but he was unable to find any associated tissue changes. Wail, Popon and Prjadko [285] injected microfilarias into crows and observed marked damage of ganglion cells in various areas, with chromatolysis, pyknosis, lipoid

281. Mya, T.: Indian M. Gaz. **63**:636, 1928.

282. Manson, P.: Tropical Diseases, Paris, C. Naud, 1904.

283. Anderson, J.: Clinical, Pathological and Therapeutic Investigations (Filariasis in British Guiana), Research Memoir 7, London School of Tropical Medicine, 1924, vol. 5.

284. Rodenwaldt, E.: Arch. f. Schiffs- u. Tropen-Hyg. **10**:389, 1908.

285. Wail, S. S.; Popon, P., and Prjadko, F.: Virchows Arch. f. path. Anat. **259**:642, 1926.

degeneration, fragmentation of fibrillae and disappearance of cell outlines. Glial nodules surrounded the destroyed ganglion cells. Microfilarias were seen to fill the capillaries but not to cause obstruction. They stressed the need for investigation of human material.

Filariasis due to Dipetalonema perstans is characterized by absence of consistent symptoms. The adult worms prefer the serous cavities and the mesentery of the body, and the microfilarias are found more often in the larger vessels than in the peripheral blood. Most writers have thought that this parasite is nonpathogenic, while others have stated that it may produce symptoms similar to those seen in Wuchereria infection. Külz [286] described a case in which the patient had typical psychic and motor symptoms of sleeping sickness, but the spinal fluid contained only microfilarias. Chambon [287] reported on a case in which the cerebrospinal fluid showed both microfilarias and trypanosomes. He surmised that the meninges were altered by the trypanosomal infection, permitting the entrance of the microfilarias. Rodhain [288] noted microfilarias in the choroid plexus in 1 case.

Loiasis, the form of filariasis caused by Loa loa, is distinguished by transient swellings and inflammatory phenomena in the subcutaneous tissues of the body, including the eye. It is possible in this infection as in the others for the microfilarias to lodge in the brain and cause symptoms.

The fourth filarial disease, onchocerciasis, is characterized by subcutaneous nodule formation and by absence of circulating microfilarias. The symptoms are due to the nodules and include local discomfort, inflammatory changes and erysipelas, and ocular disturbances. Robles [289] found among 500 patients 4 in whom the nodules had eroded both tables of the skull, with one nodule resting directly on the meninges. One patient had epileptiform seizures from irritation caused by a nodule which had penetrated the cranium. The microfilarias can apparently give rise to symptoms in this disease as in the others. Mira [290] found microfilarias in the optic nerve following enucleation of an eye for extensive ocular onchocerciasis, and Rodhain [288] observed them in the meninges in the case which he reported.

Schistosomiasis.—Schistosomiasis includes the group of diseases caused by species of flukes which inhabit the venous system of man in various tropical and subtropical countries. The three common species are Schistosoma haematobium, Schistosoma mansoni and Schistosoma japonicum. The first, S. haematobium, is endemic throughout Africa

286. Külz: Arch. f. Schiffs- u. Tropen-Hyg. **12**:547, 1908.
287. Chambon, M.: Bull. Soc. path. exot. **26**:613, 1933.
288. Rodhain, J.: Tr. Roy. Soc. Trop. Med. & Hyg. **30**:501, 1936.
289. Robles, R.: Bull. Soc. path. exot. **12**:442, 1919.
290. Mira, M. G.: Riforma med. **50**:858, 1934.

and the Near East. It usually localizes in veins of the pelvic plexus, particularly in the wall of the bladder, where the extruded ova cause symptoms of hematuria, dysuria, cystitis and other urinary difficulties. S. mansoni is found in South America and the Antilles in addition to the valley of the Nile River and other parts of Africa. It commonly localizes in the portal system and gives rise to chronic dysentery and various intestinal symptoms. Ova are found in great numbers in the liver, causing cirrhosis with splenomegaly. S. japonicum is found in the Far East, in China, Japan, Formosa, Celibes and the Philippines. It localizes in the portal system as does S. mansoni, and similar symptoms are produced, though they are often of a more severe nature.

The infection is a chronic one, and parasites may live in the body for many years. In some areas, the disease is severe, and a large percentage of the population is infected. Persons exposed to repeated infections are most likely to have chronic sequelae.

There are two ways in which the central nervous system is affected in schistosomiasis, according to Greenfield and Pritchard.[291] In one lesions are caused by the circulating ova which get past the barrier of the liver and the lung and act as emboli in the brain. In the other, the circulating larvae develop into adults in veins of the brain instead of in the portal system, and ova are extruded in situ. Though no adult worms have been found in such an aberrant location, this possibility was suggested to the authors on the basis of the large numbers of eggs circumscribed within the lesions which they observed. In the first of 2 cases which they reported the symptoms consisted of epileptiform seizures, hemianopia, scotomas, aphasia, clumsiness of the right hand and difficulty in thinking. The findings included injected optic disks, slight weakness of the right side of the face, increased reflexes on the right side, and a spinal fluid with 79 cells—93 per cent lymphocytes. A tumor was removed from just beneath the surface of the brain in the left parietal region, which proved to be a granuloma containing eggs of S. japonicum. In their second case the symptoms were persistent headaches and vomiting, scotomas, a field defect and difficulty of speech and finer movements of the right hand. A large tumor was similarly removed from the left parieto-occipital region. The pathologic observations were the same in both cases. Grossly the tumors were yellowish and friable. Cut sections revealed numerous small areas resembling minute abscesses with caseous contents, not unlike the picture seen in tuberculoma. Microscopically these areas appeared as foci of degenerated cells with groups of 10 to 20 schistosomal eggs in the center, and scattered eggs about the periphery. Some of these single ova were surrounded by a zone of granules with crenated margins. The abscess

291. Greenfield, J. G., and Pritchard, B.: Brain **60**:361, 1937.

areas were surrounded by, first, a zone of fibroblasts and endothelial cells, with occasional giant cells, lymphocytes and plasma cells. This zone contained many small blood vessels and occasionally masses of endothelial cells. There was a gradual change to normal brain tissue, but for a considerable distance all the vessels were heavily infiltrated with mononuclear cells.

Other authors have believed that the lesions of the nervous system were due to embolic ova. Tsunoda and Shimamura [292] in 1906 described a case in which a disturbance of speaking and tremors of the arms and legs were noted for several months. Later the patient suffered from headaches, loss of memory, changes in reflexes, epileptiform seizures and right hemiplegia. On examination the authors found numerous sclerotic nodules in the cortex and the white matter and a walnut-sized area of softening in the region of the internal capsule. Ova of S. japonicum were noted in these areas embedded in dense neuroglia or surrounded by foreign body tubercles with necrosis and destruction of tissue. There was a general increase of neuroglia, especially along the small vessels, and fiber degeneration in the right pyramidal tract was noted. Eggs were also found in the choroid plexus and the spinal cord. Ferguson [293a] reported finding the eggs of S. haematobium in both the brain and the spinal cord. In 1 case, the clinical symptoms resembled those of multiple sclerosis and the postmortem examination showed eggs in the spinal cord. The usual pathologic picture was that of a completely calcified egg surrounded by well marked signs of neuroglial hypertrophy. Edgar [294] observed a case in which convulsions and symptoms similar to those in the cases of Greenfield and Pritchard [291] were present. At operation a sharply outlined yellowish tumor, an inch in diameter, consisting of ova of S. japonicum, was found under the parietal bone. Vitug, Cruz and Bautista [295] described a case with sudden onset of convulsions in which eggs of S. japonicum were found in various tissues, including the brain. Here they were found in granular areas in the pia-arachnoid, the cortex and the white matter and in the capillaries of the choroid plexus. The brain showed formation of pseudotubercles with multinucleated giant cells about nests of eggs. There were apparent diffuse increase of glial tissue and fibrosis.

Involvement of the spinal cord by schistosome ova with production of transverse myelitis was first observed by Ferguson.[293b] Müller and

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292. Tsunoda, T., and Shimamura, S.: Wien. med. Wchnschr. **56**:1681, 1906.

293. Ferguson, A. R.: (*a*) Glasgow M. J. **79**:4, 1913; (*b*) J. Roy. Army M. Corps **29**:57, 1917.

294. Edgar, W. H.: J. Roy. Nav. M. Serv. **22**:150, 1936.

295. Vitug, W.; Cruz, J. R., and Bautista, L. D.: J. Philippine Islands M. A. **21**:291, 1941.

Stender [296] described the pathologic changes in a case of schistosomiasis with transverse myelitis beginning in the upper thoracic part of the cord. They noted numerous pseudotubercles scattered throughout the cord, most frequently in the anterior horns and in the lumbar and lower thoracic segments of the cord. A typical pseudotubercle contained an egg of S. mansoni at the center, but many did not. Near every egg was a giant cell, and in some cases giant cells completely surrounded the egg. A zone of necrotic tissue surrounded the ovum, and outside of this was a border of lymphocytes and plasma cells. Destruction of tissue, with production of glial cells and of macrophages and fat granule cells, surrounded the pseudotubercles. The ganglion cells suffered little change. The spinal fluid showed an increase of cells and a marked increase of protein. The authors surmised that the necrotic zone about the egg was caused by a toxin liberated by the egg. They also surmised that the eggs reached the cord through venous anastomoses between the portal veins and the vertebral plexus of veins. Day and Kenawy [297] recorded a case of myelitis caused by S. haematobium. Ova were found in the lumbar enlargement in the anterior horns and the lateral columns, surrounded by granulation tissue made up of histiocytes and lymphocytes. The nerve cells near the ova showed varying degrees of degeneration, possibly due to diffusion of toxins from the eggs. Similar cases were reported by Bayoumi [298] and Espin.[299]

Paragonimiasis.—Paragonimiasis is caused by an infection with the fluke Paragonimus westermani, which inhabits the tissues of the lungs of man and various mammals. It is present throughout the Orient and has also been recorded from parts of Africa and South America. In some districts of Japan 40 to 50 per cent of the population is infected. Two intermediate hosts are required in the life cycle, snails and then crabs and crayfish. Human infection is acquired through eating crayfish containing encysted larvae.

Not infrequently the larvae migrate and develop in other tissues, including the brain. Cysts similar to those found in the lungs develop in the intracranial cavity, and symptoms of an intracranial neoplasm result. The first of a number of such cases was described by Otani in 1887. His patient had lung fluke infection complicated by epileptiform seizures, apathy and confusion. Autopsy revealed a mass as large as a hen's egg in the right frontal lobe and a smaller area grown to the dura in the occipital lobe. Cut sections revealed a mass of communicating cysts

296. Müller, H. R., and Stender, A.: Arch. f. Schiffs- u. Tropen-Hyg. **34**:527, 1930.

297. Day, H. B., and Kenawy, M. R.: Tr. Roy. Soc. Trop. Med. & Hyg. **30**:223, 1936.

298. Bayoumi, M. L.: J. Egyptian M. A. **22**:457, 1939.

299. Espin, J.: Rev. Policlín. Caracas **10**:245, 1941.

containing dark brown fluid. A fluke was seen in the fluid and another in normal brain tissue. Yamigawa [300] reported a similar case with convulsions and hemiparesis of two years' duration. Pathologic examination revealed groups of fluke eggs in the cortex surrounded by nodules consisting of an atrophic center and a surrounding round cell infiltration. The surrounding vessels were frequently increased and showed proliferation of their walls. Giant cells were also present. Taniguchi [301] described the case of a 17 year old girl with symptoms of epilepsy, chorea and athetosis, weakness of the left side and intellectual deterioration. Cut sections revealed in the right frontoparietal and temporal lobes several groups of cysts filled with thick brownish liquid containing fluke eggs. The microscopic examination revealed nodules and cyst walls of two layers—an inner, somewhat fibrous layer and an outer one of dense round cell infiltration. A diffuse glial increase was noted in the surrounding normal brain tissue, and the involved pyramidal tract showed degeneration. Musgrave [302] reported on a case of epilepsy in which the dura was thickened and contained parasitic abscesses similar to those found in the lung. A few eggs were seen in the choroid plexus also.

In 1921 Kawamura and Yamaguchi [303] reviewed 38 cases of cerebral paragonimiasis in the literature and added 37 of their own. With regard to children, they stressed the occurrence of epileptiform seizures, transient paralyses and other neurologic disturbances. In 1 case which they examined pathologically the patient had a nine year history of convulsions and deterioration. Several nodules were seen on the surface of the brain, and cut sections revealed numerous cystlike nodules and areas of softening in the cortical layers. The cysts were encapsulated by a fibrous membrane, and the contents contained lime granules, cholesterol crystals, eggs, giant cells and amorphous material. Round cell proliferation and increased glial cells surrounded the capsule. No flukes were found. Kimura [304] reported on a case in which mental peculiarities and hallucinations were noted before death. Autopsy revealed many cysts throughout the brain with atrophy of the occipital lobe.

Yokogawa and Suyemori [305] studied the infection experimentally and concluded that the main pathologic changes were probably due to flukes migrating in and out of the brain by way of the loose connective tissue in the neck.

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300. Yamigawa, K.: Virchows Arch. f. path. Anat. **119**:447, 1890.
301. Taniguchi: Arch. f. Psychiat. **38**:100, 1904.
302. Musgrave, W. E.: Philippine J. Sc. **2**:15, 1907.
303. Kawamura, R., and Yamaguchi, M.: Japan M. World **1**:1, 1921.
304. Kimura, O.: Mitt. a. d. Path. Inst. d. k. Univ. zu Sendai **1**:1, 1919.
305. Yokogawa, S., and Suyemori, S.: Am. J. Hyg. **1**:63, 1921.

Book Reviews

Physical Chemistry of Cells and Tissues. By Rudolf Höber, M.D., department of physiology, University of Pennsylvania, Philadelphia. With the collaboration of: David I. Hitchcock, Yale University Medical School; J. B. Bateman, Mayo Clinic, Rochester, Minn.; David R. Goddard, University of Rochester, and Wallace O. Fenn, University of Rochester. Price $9. Pp. 676 with 70 illustrations. Philadelphia: The Blakiston Company, 1945.

This is a difficult book to characterize. It succeeds *"Physikalische Chemie der Zelle und der Gewebe"* by the distinguished senior author of the present volume, now of the University of Pennsylvania, but is only distantly related to it. The book does not purport to be a systematic review of physical chemistry as applied to the problems of cells and tissues, as its predecessor was, but is devoted rather to an attempt to bring certain problems of general physiology into closer relationship with physical-chemical science, the authors having deliberately chosen "a new start from a new base line." The volume suffers from the lack of integration common to books by multiple authors. This is in part attributable to incompleteness, since, according to the preface, it was impossible, owing to the war, to carry out the original plan in its entirety.

The first 216 pages of a total of 635 pages of text are devoted to a review of certain aspects of physical chemistry. Section 1, by David I. Hitchcock, is concerned with "Selected Principles of Physical Chemistry." Section 2, by J. R. Bateman, treats of "Large Molecules; Their Physicochemical Properties and Their Architectural and Functional Significance in Living Matter." Of the remaining six sections, four are contributed by Dr. Höber. Three of these, including one which is a short introduction, are concerned primarily with permeability: section 4, with the permeability of cells; section 8, with studies of permeability as applied to the functions of tissues, such as intestinal absorption, the formation of urine and the elaboration of gastric juice. Section 5 deals with the effects on cells of experimental changes in their environment, and is also largely concerned with permeability. Interpolated between section 5 and section 8, the reason for this arrangement not being clear to the reviewer, are section 6, by David R. Goddard, entitled "The Respiration of Cells and Tissues," and section 7, by Wallace O. Fenn, entitled "Contractility."

As stated in the introduction to section 1, "the treatment of physicochemical principles is based on the assumption that its readers already have some knowledge of the subject." Inspection of the first few pages of the text will reveal that this implies a considerable working knowledge of the calculus, differential and integral. The book is definitely not for beginners. Not only is the approach to physical chemistry somewhat advanced, but the reader is largely left to perceive for himself the applicability of the facts presented. Also the "selected principles" omit certain subjects, such as surface phenomena, which could have been reviewed with profit.

The remainder of the book falls into a series of three monographs, those listed above by Drs. Goddard and Fenn and a longer one, chiefly on the subject of permeability, by Dr. Höber. As to the section by Dr. Fenn, it may be said quite simply that it is of the excellent quality one has learned to expect from this author. It will be of interest to every student of general physiology and is integrated cellular physiology at its best. Being largely qualitative in its treatment, it may be read as an introduction to its subject matter and to the book itself. Dr. Goddard deals chiefly with intermediate carbohydrate metabolism. He has invoked physical-chemical principles whenever this was necessary, and in this way his section gives meaning to some parts of section 1. His presentation is excellent in every way and fulfils the promise of the title of the book. The reader would perhaps gain

the utmost from the book by reading sections 6 and 7 first, referring back to sections 1 and 2 when he feels the need of consulting a more detailed presentation of physical-chemical principles.

Dr. Höber's sections, viewed as a monograph, suffer from a lack of orientation to a unified concept of permeability. In fact, as the author states, the term "permeability" is used in section 8 in a sense (actually two senses) different from that (actually those) used in the earlier sections, and it is not always clear which sense is intended. The material on the subject of permeability is here for the student of the subject, although not, without considerable effort, for the uninitiated. The final chapter of the book, which is part of Dr. Höber's contribution, is a timely treatment of the energetics of active transfer, the transferring devices and their mechanics.

As Dr. Höber points out in the preface, "during the last four decades a tremendous revolution in our conception of the inorganic world has taken place, which during the last twenty years has progressively seized the aspect of the world of organisms." This book represents a noteworthy attempt to reorient certain fields of general physiology to the newer physics and physical chemistry, and as such will be of interest to biologists generally.

Pulmonary Edema and Inflammation: An Analysis of Processes Involved in the Formation and Removal of Pulmonary Transudates and Exudates. By Cecil K. Drinker, M.D., D.Sc., professor of physiology, Harvard University School of Public Health, Boston. Cloth. Pp. 106, with 27 figures and illustrations. Price, $2.50. Cambridge, Mass.: Harvard University Press, 1945.

This monograph is composed of the Nathalie Gray Bernard Lectures delivered at the Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C., in December 1944, together with a fifth chapter on artificial respiration. It contains experimental results obtained by the author and his students, some of which have not previously been published. The chapters take up in succession the relation of lung structure to edema and inflammation, physiologic factors in pulmonary edema and inflammation, breathing movements and pulmonary edema, preventive and therapeutic measures in asphyxiating pulmonary disease, and artificial respiration.

The book is concerned more with pulmonary edema than with inflammation, and in inflammation the fluid component of the exudate is stressed to the exclusion of the cellular phenomena. Increased permeability of capillary endothelium, caused by anoxia, is considered more important in the development of pulmonary edema than are changes in pulmonary capillary pressure. A thiourea derivative which selectively affects the endothelium of the capillaries of the lung was also used to produce pulmonary edema. It is hoped that the withholding of the name of this important compound is explained by the exigencies of war. This book is highly recommended to all who are concerned with normal and abnormal states in the lung.

Notes and News

Appointments.—Francis C. Tucker has been appointed assistant pathologist at St. Luke's Hospital, Chicago.

Lawrence W. Smith has resigned as professor of pathology in the Temple University School of Medicine, Philadelphia.

Philip R. White, since 1938 associate in the department of animal and plant pathology of the Rockefeller Institute of Princeton, N. J., has become a member of the institute for cancer research at Lankenau Hospital, Philadelphia.

Arthur C. Allen, who for the past three and a half years has served as pathologist at the Army Institute of Pathology, Washington, D. C., has recently been appointed associate professor of pathology at New York Medical College.

Crichton McNeal, stationed at the Army Institute of Pathology, Washington, D. C., has been appointed instructor in pathology at the University of Utah.

Dr. V. D. Sneeden has been released from military service and has returned to the department of pathology of the University of Oregon Medical School as associate professor.

Ida A. Bengston and Alice C. Evans, bacteriologists of the United States Public Health Service, have retired after many years of work in the National Institute of Health.

Deaths.—Newton G. Evans, professor of pathology in the College of Medical Evangelists since 1914 and for many years pathologist of the Los Angeles County Hospital, died Dec. 19, 1945, at the age of 71.

Henry B. Ward, professor emeritus of zoology in the University of Illinois, well known for his work in parasitology, died Nov. 30, 1945, at the age of 80 years.

Society News.—The Association of Pathologists of West Virginia has been organized with C. C. Fenton, Morgantown, as president and W. T. McClure, Wheeling, as secretary-treasurer. The first meeting will be held at Huntington, W. Va., May 14, 1946, in conjunction with the State Medical Association.

The American College of Physicians will hold its 1946 meeting in Philadelphia, May 13 to 17 inclusive, at the Municipal Auditorium.

The American Association of Pathologists and Bacteriologists will meet at the University of Chicago, Friday and Saturday, March 8 and 9, 1946.

The Federation of American Societies for Experimental Biology will meet in Atlantic City, N. J., beginning on Monday, March 11, 1946.

The thirty-seventh annual meeting of the American Association for Cancer Research will be held in Atlantic City, N. J., Monday and Tuesday, March 11 and 12, 1946, concurrently with the opening meetings of the Federation of American Societies for Experimental Biology. The Ambassador Hotel will be headquarters.

Awards.—Wendell M. Stanley, of the Rockefeller Institute of Princeton, N. J., has been awarded the 1946 William H. Nichols Medal of the New York Section of the American Chemical Society in recognition of his work on the chemistry of viruses.

The Copley Medal of the Royal Society has been awarded to O. T. Avery, of the Rockefeller Institute of New York, for his introduction of chemical methods in the study of anti-infectious immunity.

Postgraduate Refresher Course for Pathologists Returning from Military Service.—The American Society of Clinical Pathologists announces a postgraduate refresher course for pathologists returning from military service, to be held in Chicago, at the Drake Hotel, March 2 to 5, 1946.